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Practitioner's Docket No. P-1027

CHAPTER II

Preliminary Classification:

Proposed Class:

Subclass:

NOTE: *"All applicants are requested to include a preliminary classification on newly filed patent applications. The preliminary classification, preferably class and subclass designations, should be identified in the upper right-hand corner of the letter of transmittal accompanying the application papers, for example 'Proposed Class 2, subclass 129.'" M.P.E.P., § 601, 7th ed.*

TRANSMITTAL LETTER TO THE UNITED STATES ELECTED OFFICE (EO/US)

(ENTRY INTO U.S. NATIONAL PHASE UNDER CHAPTER II)

PCT/EP 99/05711	06 August 1999	30 October 1998
INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED

MICROBIAL ACTIVATION OF LAYER SILICATES

TITLE OF INVENTION

Christian Fabry; Dr. Stefan Dick; Dr. Werner Zschau

APPLICANT(S)

Box PCT
Assistant Commissioner for Patents
Washington D.C. 20231

ATTENTION: EO/US

CERTIFICATION UNDER 37 C.F.R. § 1.10*

*(Express Mail label number is **mandatory**.)*
(Express Mail certification is optional.)

I hereby certify that this Transmittal Letter and the papers indicated as being transmitted therewith is being deposited with the United States Postal Service on this date April 26, 2001, in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number EK985526267US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Dorothy Goodlett

(type or print name of person mailing paper)

Dorothy Goodlett

Signature of person mailing paper

WARNING: *Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. § 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.*

***WARNING:** Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. § 1.10(b).*
*"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.*

NOTE: To avoid abandonment of the application, the applicant shall furnish to the USPTO, not later than 20 months from the priority date: (1) a copy of the international application, unless it has been previously communicated by the International Bureau or unless it was originally filed in the USPTO; and (2) the basic national fee (see 37 C.F.R. § 1.492(a)). The 30-month time limit may not be extended. 37 C.F.R. § 1.495.

WARNING: *Where the items are those which can be submitted to complete the entry of the international application into the national phase are subsequent to 30 months from the priority date the application is still considered to be in the international state and if mailing procedures are utilized to obtain a date the express mail procedure of 37 C.F.R. § 1.10 must be used (since international application papers are not covered by an ordinary certificate of mailing—See 37 C.F.R. § 1.8.*

NOTE: Documents and fees must be clearly identified as a submission to enter the national state under 35 U.S.C. § 371 otherwise the submission will be considered as being made under 35 U.S.C. § 111. 37 C.F.R. § 1.494(f).

I. Applicant herewith submits to the United States Elected Office (EO/US) the following items under 35 U.S.C. § 371:

a. ☒ This express request to immediately begin national examination procedures (35 U.S.C. § 371(f)).

b. ☒ The U.S. National Fee (35 U.S.C. § 371(c)(1)) and other fees (37 C.F.R. § 1.492) as indicated below:

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2. Fees

CLAIMS FEE	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS
☐*	TOTAL CLAIMS 25	−20=	5	× $18.00=	$ 90.00
	INDEPENDENT CLAIMS 1	−3=		× $80.00=	
	MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	
BASIC FEE**	☐ U.S. PTO WAS INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where an International preliminary examination fee as set forth in § 1.482 has been paid on the international application to the U.S. PTO: ☐ and the international preliminary examination report states that the criteria of novelty, inventive step (non-obviousness) and industrial activity, as defined in PCT Article 33(1) to (4) have been satisfied for all the claims presented in the application entering the national stage (37 C.F.R. § 1.492(a)(4)) ..... $100.00 ☐ and the above requirements are not met (37 C.F.R. § 1.492(a)(1)) ..... $690.00 ☑ U.S. PTO WAS NOT INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where no international preliminary examination fee as set forth in § 1.482 has been paid to the U.S. PTO, and payment of an international search fee as set forth in § 1.445(a)(2) to the U.S. PTO: ☐ has been paid (37 C.F.R. § 1.492(a)(2)) ..... $710.00 ☐ has not been paid (37 C.F.R. § 1.492(a)(3)) ..... $1000.00 ☑ where a search report on the international application has been prepared by the European Patent Office or the Japanese Patent Office (37 C.F.R. § 1.492(a)(5)) $860.00				$860.00
	Total of above Calculations				= $950.00
SMALL ENTITY	Reduction by 1/2 for filing by small entity, if applicable. Affidavit must be filed also. (note 37 C.F.R. § 1.9, 1.27, 1.28)				–
	Subtotal				
	Total National Fee				$ 950.00
	Fee for recording the enclosed assignment document $40.00 (37 C.F.R. § 1.21(h)). (See Item 13 below). See attached "ASSIGNMENT COVER SHEET".				40.00
TOTAL	Total Fees enclosed				$ 990.00

*See attached Preliminary Amendment Reducing the Number of Claims.

☑ Attached is a ☑ check ☐ money order in the amount of $ 990.00

☑ Authorization is hereby made to charge ~~the amount of $~~ _____

☑ to Deposit Account No. 03-3420

☐ to Credit card as shown on the attached credit card information authorization form PTO-2038.

WARNING: *Credit card information should* ***not*** *be included on this form as it may become public.*

☑ Charge any additional fees required by this paper or credit any overpayment in the manner authorized above.

A duplicate of this paper is attached.

WARNING: *"To avoid abandonment of the application the applicant shall furnish to the United States Patent and Trademark Office not later than the expiration of 30 months from the priority date: * * * (2) the basic national fee (see § 1.492(a)). The 30-month time limit may not be extended." 37 C.F.R. § 1.495(b).*

WARNING: *If the translation of the international application and/or the oath or declaration have not been submitted by the applicant within thirty (30) months from the priority date, such requirements may be met within a time period set by the Office. 37 C.F.R. § 1.495(b)(2). The payment of the surcharge set forth in § 1.492(e) is required as a condition for accepting the oath or declaration later than thirty (30) months after the priority date. The payment of the processing fee set forth in § 1.492(f) is required for acceptance of an English translation later than thirty (30) months after the priority date. Failure to comply with these requirements will result in abandonment of the application. The provisions of § 1.136 apply to the period which is set. Notice of Jan. 3, 1993, 1147 O.G. 29 to 40.*

3. ☑ A copy of the International application as filed (35 U.S.C. § 371(c)(2)):

NOTE: Section 1.495 (b) was amended to require that the basic national fee and a copy of the international application must be filed with the Office by 30 months from the priority date to avoid abandonment. "The International Bureau normally provides the copy of the international application to the Office in accordance with PCT Article 20. At the same time, the International Bureau notifies applicant of the communication to the Office. In accordance with PCT Rule 47.1, that notice shall be accepted by all designated offices as conclusive evidence that the communication has duly taken place. Thus, if the applicant desires to enter the national stage, the applicant normally need only check to be sure the notice from the International Bureau has been received and then pay the basic national fee by 30 months from the priority date." Notice of Jan. 7, 1993, 1147 O.G. 29 to 40, at 35-36. See item 14c below.

a. ☐ is transmitted herewith.

b. ☑ is not required, as the application was filed with the United States Receiving Office.

c. ☑ has been transmitted

i. ☑ by the International Bureau.
Date of mailing of the application (from form PCT/1B/308):
_____.

ii. ☐ by applicant on _____. *(Date)*

4. ☑ A translation of the International application into the English language (35 U.S.C. § 371(c)(2)):

a. ☑ is transmitted herewith.

b. ☐ is not required as the application was filed in English.

c. ☐ was previously transmitted by applicant on _____. *(Date)*

d. ☐ will follow.

5. ☐ Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. § 371(c)(3)):

NOTE: The Notice of January 7, 1993 points out that 37 C.F.R. § 1.495(a) was amended to clarify the existing and continuing practice that PCT Article 19 amendments must be submitted by 30 months from the priority date and this deadline may not be extended. The Notice further advises that: "The failure to do so will not result in loss of the subject matter of the PCT Article 19 amendments. Applicant may submit that subject matter in a preliminary amendment filed under section 1.121. In many cases, filing an amendment under section 1.121 is preferable since grammatical or idiomatic errors may be corrected." 1147 O.G. 29-40, at 36.

a. ☐ are transmitted herewith.

b. ☐ have been transmitted

i. ☐ by the International Bureau.
Date of mailing of the amendment (from form PCT/1B/308):
_____.

ii. ☐ by applicant on _____. *(Date)*

c. ☐ have not been transmitted as

i. ☐ applicant chose not to make amendments under PCT Article 19.
Date of mailing of Search Report (from form PCT/ISA/210.):
_____.

ii. ☐ the time limit for the submission of amendments has not yet expired. The amendments or a statement that amendments have not been made will be transmitted before the expiration of the time limit under PCT Rule 46.1.

6. ☐ A translation of the amendments to the claims under PCT Article 19 (38 U.S.C. § 371(c)(3)):

a. ☐ is transmitted herewith.

b. ☐ is not required as the amendments were made in the English language.

c. ☐ has not been transmitted for reasons indicated at point 5(c) above.

7. ☑ A copy of the international examination report (PCT/IPEA/409)

☑ is transmitted herewith.

☐ is not required as the application was filed with the United States Receiving Office.

8. ☐ Annex(es) to the international preliminary examination report

a. ☐ is/are transmitted herewith.

b. ☐ is/are not required as the application was filed with the United States Receiving Office.

9. ☐ A translation of the annexes to the international preliminary examination report

a. ☐ is transmitted herewith.

b. ☐ is not required as the annexes are in the English language.

10. ☑ An oath or declaration of the inventor (35 U.S.C. § 371(c)(4)) complying with 35 U.S.C. § 115

a. ☐ was previously submitted by applicant on _____.
Date

b. ☑ is submitted herewith, and such oath or declaration

i. ☑ is attached to the application.

ii. ☐ identifies the application and any amendments under PCT Article 19 that were transmitted as stated in points 3(b) or 3(c) and 5(b); and states that they were reviewed by the inventor as required by 37 C.F.R. § 1.70.

c. ☐ will follow.

II. Other document(s) or information included:

11. ☑ An International Search Report (PCT/ISA/210) or Declaration under PCT Article 17(2)(a):

a. ☑ is transmitted herewith.

b. ☐ has been transmitted by the International Bureau.
Date of mailing (from form PCT/IB/308): _____.

c. ☐ is not required, as the application was searched by the United States International Searching Authority.

d. ☐ will be transmitted promptly upon request.

e. ☐ has been submitted by applicant on _____.
Date

12. ☑ An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98:

a. ☐ is transmitted herewith.

Also transmitted herewith is/are:

☐ Form PTO-1449 (PTO/SB/08A and 08B).

☐ Copies of citations listed.

b. ☑ will be transmitted within THREE MONTHS of the date of submission of requirements under 35 U.S.C. § 371(c).

c. ☐ was previously submitted by applicant on _____.
Date

13. ☑ An assignment document is transmitted herewith for recording.

A separate ☑ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or ☐ FORM PTO 1595 is also attached.

Assigning to: Süd-Chemie AG
Lenbachplatz 6
D-80333 Munchen
Fed. Republic of Germany

14. ☑ Additional documents:

a. ☐ Copy of request (PCT/RO/101)

b. ☐ International Publication No. _____

i. ☐ Specification, claims and drawing

ii. ☐ Front page only

c. ☑ Preliminary amendment (37 C.F.R. § 1.121)

d. ☐ Other

_____

_____

_____

15. ☑ The above checked items are being transmitted

a. ☑ before 30 months from any claimed priority date.

b. ☐ after 30 months.

16. ☐ Certain requirements under 35 U.S.C. § 371 were previously submitted by the applicant on _____, namely:

_____

_____

_____

_____

_____

AUTHORIZATION TO CHARGE ADDITIONAL FEES

WARNING: *Accurately count claims, especially multiple dependant claims, to avoid unexpected high charges if extra claims are authorized.*

NOTE: "A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).

NOTE: "Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).

☑ Please charge, in the manner authorized above, the following additional fees that may be required by this paper and during the entire pendency of this application:

☑ 37 C.F.R. § 1.492(a)(1), (2), (3), and (4) (filing fees)

WARNING: *Because failure to pay the national fee within 30 months without extension (37 C.F.R. § 1.495(b)(2)) results in abandonment of the application, it would be best to always check the above box.*

☑ 37 C.F.R. § 1.492(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 C.F.R. § 1.492(d)), it might be best not to authorize the PTO to charge additional claim fees, except possible when dealing with amendments after final action.

☐ 37 C.F.R. § 1.17 (application processing fees)

☐ 37 C.F.R. § 1.17(a)(1)–(5) (extension fees pursuant to § 1.136(a).

☐ 37 C.F.R. § 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. § 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 C.F.R. § 1.311(b).

NOTE: 37 C.F.R. § 1.28(b) requires "Notification of any change in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying . . . issue fee." From the wording of 37 C.F.R. § 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

☐ 37 C.F.R. § 1.492(e) and (f) (surcharge fees for filing the declaration and/or filing an English translation of an International Application later than 30 months after the priority date).

SIGNATURE OF PRACTITIONER

Reg. No.: 31,945

Scott R. Cox

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(type or print name of practitioner)

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Customer No.:

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Louisville, KY 40202

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of Fabry, Christian, et al.	:	
	:	Art Unit:
Serial No.	:	
	:	Examiner:
Filing Date:	:	
	:	
Attorney Docket No. P-1027	:	
	:	
For: MICROBIAL ACTIVATION OF LAYER SILICATES	:	

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

In the Claims

The translation of the priority document (Exhibit A) which is submitted as the application contains two sets of claims: a first set on pages 21 and 22 (Claims 1-17) and a second set on pages 24 and 25 (numbered Claims 1-16). Please cancel all of these claims and add new Claims 17-41 which are attached as Exhibit B to this Preliminary Amendment.

In the Specification

Attached as Exhibit C is a copy of the translation of the Specification with handwritten amendments noted therein.

Exhibit D is a clean copy of the specification incorporating the amendments to the translation of the specification and new Claims 17-41. The new Claims 17-41 are on pages 21-23. Finally, the Abstract page has been renumbered as page 24.

Discussion

The applicants have amended the original specification (Exhibit A) in the manner shown in Exhibit C. After incorporating these changes into the specification, the applicants offer Exhibit D as the amended specification for review by the United States Patent and Trademark Office.

CONCLUSION

The applicants believe all claims are now in condition for review.

Respectfully submitted,

Scott R. Cox
Reg. No. 31,945
LYNCH, COX, GILMAN & MAHAN, P.S.C.
400 West Market Street, Suite 2200
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Attachments: Exhibits A-D

We Claim

17. A process for the activation of a layered silicate for treatment of oils, fats and waxes comprising

preparing a layered silicate composition,

activating that layered silicate composition by treating the layered silicate composition with an acid-producing microorganism.

18. The process of Claim 17 wherein the layered silicate comprises a smectite clay.

19. The process of Claim 17 wherein the layered silicate comprises a montmorillonite clay.

20. The process of Claim 19 wherein the montmorillonite clay comprises a bentonite clay.

21. The process of Claim 17 wherein the layered silicate comprises a palygorskite clay.

22. The process of Claim 20 wherein the layered silicate further comprises a palygorskite clay.

23. The process of Claim 17 wherein the acid-producing microorganism comprises a sulfur-oxidizing bacteria.

24. The process of Claim 17 wherein the acid-producing microorganism comprises an iron-oxidizing bacteria.

25. The process of Claim 23 wherein the sulfur-oxidizing bacteria comprises Thiobacillus thiooxidans.

26. The process of Claim 24 wherein the iron-oxidizing

bacteria comprises Thiobacillus ferrooxidans.

27. The process of Claim 17 wherein the acid-producing microorganism produces citric acid.

28. The process of Claim 27 wherein the citric acid-producing microorganism comprises Aspergillus niger.

29. The process of Claim 17 further comprising breaking up the layered silicate composition prior to activation into clumps with a size from about 0.5 cm to about 5 cm.

30. The process of Claim 17 further comprising adding the acid-producing microorganisms to an inoculant material prior to activating the layered silicate composition with the microorganisms which have been added to the inoculant material.

31. The process of Claim 30 wherein the population of the microorganisms added to the layered silicate is from about 10^2 to about 10^{10} bacteria/g of the inoculant material.

32. The process of Claim 17 further comprising maintaining the temperature of the layered silicate composition during activation within the range from about 20 to about 35°C.

33. The process of Claim 17 further comprising maintaining the water content of the layered silicate composition during the activating process within a range from about 15 percent by weight to about 70 percent by weight.

34. The process of Claim 30 wherein the inoculant material added to the layered silicate comprises about 5 to about 20 percent

of the overall composition after the inoculant material has been added.

35. The process of Claim 17 further comprising mixing and aerating the layered silicate composition while it is being activated with the acid-producing microorganism.

36. The process of Claim 35 wherein the activation process occurs for a period of time from about 1 to about 365 days.

37. The process of Claim 17 further comprising adding nutrients for the microorganisms to the layered silicate composition prior to activation.

38. The process of Claim 37 wherein the nutrients added comprise sulfur-containing products.

39. The process of Claim 17 further comprising adding small quantities of a dilute acid to the layered silicate composition prior to activation with the acid-producing microorganisms.

40. An activated layered silicate prepared by the process of Claim 17.

41. A process for decolorizing oils, fats or waxes comprising contacting the oils, fats or waxes with the activated layered silicate prepared by the process of Claim 17.

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TRANSLATION FROM GERMAN

PATENT ATTORNEYS

R. SPLANEMANN, DIPL.-ING.; DR. B. REITZNER, DIPL.-CHEM;

K. BARONETZKY, DIPL.-ING.

AGENTS AUTHORIZED AT THE EUROPEAN PATENT OFFICE

PROFESSIONAL REPRESENTATIVES BEFORE EPO

AUTHORIZED AGENTS AT THE EUROPEAN PATENT OFFICE

Süd-Chemie AG	**80331 Munich**	
Lenbachplatz 6	**TAL 13**	October 30, 1998
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80333 Munich	FAX:	(089) 297692
	TELEX:	528418 INTUS D
	OUR DOSSIER:	4465-I-18.862
	YOUR REFERENCE:	Süd-Chemie AG

Patent application

Microbial activation of layer silicates

Specification

The invention pertains to a process for the activation of layer silicates with use being made of microorganisms.

US 1,492,184 describes the activation of raw clay with maximally 10% by weight of concentrated acid. It is preferable that a pre-dried and ground raw clay be impregnated in this

regard. Montmorillonite, bauxite, willonite, pyrophyllite, kaolinite and fuller's earth are designated as examples of "clays".

US 1,752,721 describes a process for the treatment of "earthy materials" in order to increase their adsorption properties; accordingly, a clay material is mixed with solid oxalic acid and then it is heated, in the absence of added water, in order to bring about a reaction between the oxalic acid and the clay mineral. The clay mineral is treated with approximately 1 to 5% of oxalic acid in this connection.

DE-C-304706 describes a process for increasing the decolorizing power of fuller's earth. In this process, the raw fuller's earth is stirred with the acid to give a doughy mass, and then it is dried.

A process for the treatment of clay is known from US 4,847,226 in which the clay is extruded, ground and then added to an aqueous solution of an acid in order to produce a suspension; the suspension is heated, and then the acid-treated clay is separated, washed, filtered off and dried. The intended purpose of the treatment is to improve the ability of the clay to filter out impurities from liquids. In particular, oil-soluble dyes are removed from oils.

The objective ~~that now forms the basis~~ of the invention is to provide a process for the activation of layer silicates ~~that can be carried out~~ without the addition of corrosive acids that can cause intense burns and that endanger natural water systems, whereby this process is superior to the prior art from the standpoints of operational safety and environmental protection and also from ~~the~~ an economic point of view.

Surprisingly, it has now been found that the activation of layer silicates can take place via the use of microorganisms without any addition of an acid or, as the case may be, a solution of an acid.

The use of acid-producing microorganisms for leaching out residues from low grade copper ores is already known in the prior art. In addition, the growth of such microorganisms on ores, such as pyrites, is exploited in order to assist flotation. A review of these and further applications of the treatment and processing of ores and for metal recovery is to be found in the publication by

C.L. Brierley: Bacteria as aids in mining; ~~the spectrum of this science.~~ Spektrum der Wissenschaft Industrielle Mikrobiologie, 60 (1989).

The bacterial oxidation of elemental sulfur is exploited in agriculture in order to make sulfate available to ~~the~~ plants and to make phosphate and micro-nutrient substances available as well.

The use of microorganisms for the activation of layer silicates is not known in the prior art.

~~The subject of the invention is a process in accordance with Claim 1.~~ The term "activation" is ~~to be~~ understood to mean a process for increasing the decolorizing activity of the layer silicate.

The activated layer silicates in accordance with the invention can be used, in particular, as fuller's earth materials for the treatment of oils, fats or waxes.

Glyceride oils, waxes and fats and mineral oils ~~pass~~ are passed through one or more adsorptive treatment stages during their refining ~~via~~ using inorganic adsorbents. The oil or fat that is to be treated is thereby brought into contact with an inorganic adsorbent at an elevated temperature. ~~In this connection,~~ the adsorbent ~~has the task of freeing~~ filters from the oil ~~from~~ substances that are disadvantageous in subsequent processes or for storage, such as e.g. pigments, phospholipids, materials that produce turbidity, metals, free fatty acids, oxidized compounds, etc. In order to do this, the adsorbent requires adsorptive properties, in order to permit ~~e.g.~~ the removal of phospholipids or chlorophyll materials, and catalytic properties for ~~e.g.~~ degrading dyes or peroxide compounds that are contained in the oil.

Because of their advantageous properties, especially their high specific surface area, ~~and their~~ sorption capacity and ion exchange capacity, the activated layer silicates that are prepared in accordance with the invention can also find use in other sectors.

The layer silicates that are listed in Ullmann's Encyklopädie der technischen Chemie ["Encyclopedia of industrial Chemistry"], Volume 21, pages 370-375 (1982) are included among the layer silicates that are usable in the process in accordance with the invention. In particular,

use can be made of natural and synthetic clay minerals that are capable of being activated, such as ~~e.g.~~ the smectites - including montmorillonite, beidellite, nontronite, wolchonskoite, stevensite, hectorite, swinefordite, saponite and sauconite - along with vermiculites, illites, mixed layer minerals, palygorskite (attapulgite) and sepiolite. The latter two materials are also designated as hormites. The clay minerals can be present in their H form, their alkali metal form or their alkaline earth form.

In accordance with a preferred form of embodiment in accordance with the invention, the layer silicate is a ~~triple~~ three layer silicate, e.g. a naturally occurring smectitic clay, especially a bentonite clay or a palygorskite clay or mixtures thereof.

Palygorskite clays comprise attapulgite clays that are also known as attalpulgus clays, or Georgia fuller's earths. As a rule, these clays consist primarily of the mineral attapulgite, i.e. a crystalline, hydrated magnesium aluminum silicate, but they can also contain considerable quantities of other minerals, such as e.g. bentonite (montmorillonite), calcium carbonate, quartz and feldspar and, in many cases, sepiolite. The preferred clays contain at least 10% by weight, and up to 90% by weight, of attapulgite and, preferably, up to 20 to 60% by weight thereof.

Non-calcined, naturally occurring mixtures of palygorskite clay and calcium bentonite clay are especially preferred. Such natural mixtures can contain pyrites that can serve as a substrate for sulfur-oxidizing bacteria and iron-oxidizing bacteria such as Thiobacillus ferrooxidans.

An attapulgite/bentonite mixture of clays is used in accordance with an especially preferred form of embodiment.

The microorganisms that are used for activation in accordance with the invention are bacteria, archaebacteria or fungi e.g. of the Aspergillus, Acidianus, Acidimicrobium, Acidiphilium, Acidobacterium, Acidocella, Alicyclobacillus, Leptospirillium, Metallosphaera, Picrophylus, Sarcina, Stygiolobus, Sulfobacillus, Sulfolobus, Thermoplasma, Thiobacillus and Thiomonas strains. In addition to the acid-producing bacteria that are preferred here - especially the so-called sulfuric acid bacteria - use can also be made of nitric acid bacteria and acetic acid bacteria

as well as microorganisms that produce oxalic acid, citric acid, gluconic acid or other organic acids.

The use of bacteria that oxidize pyrites is especially advantageous when the layer silicate that is used already contains pyrites, so that this nutrient substance for the bacteria does not have to be added. In addition, it has been found that some naturally occurring bentonite/attapulgite clay mixtures already naturally contain Thiobacillus ferrooxidans and Thiobacillus thiooxidans in small quantities, and these can be induced to activate the layer silicate.

The last two types of bacteria that were designated are strongly chemolithoautotrophic, i.e. their growth cannot be stimulated by providing organic materials, such as nutrient substances or vitamins. Both belong to the group of acidophilic bacteria and they prefer pH values around 2 and temperatures of around 30°C.

Use can be made of both ~~wild~~ natural type strains of the microorganisms and also strains that have been cultivated in the laboratory (e.g. Thiobacillus thiooxidans DSMZ-11478; Aspergillus niger DSMZ-823; see the DSMZ catalog, 1998). Prior cultivation of the microorganisms offers the advantage that adaptation can be carried out ~~in terms of~~ using the conditions that have been selected for the activation of the layer silicate. In addition, the microorganisms can be selected conventionally in terms of advantageous properties (e.g. rapid growth under the conditions that have been selected for activation).

In the case of using aerobic microorganisms (such as, for example, T. thiooxidans, T. ferrooxidans and A. niger), an adequate supply of oxygen has to be ensured during activation of the layer silicate. This can be ensured, for example, by regular mixing in with the layer silicate (every 1 to 7 days) and avoiding excessive compaction. The process of mixing in also encourages uniform distribution, more rapid multiplication and higher metabolic efficiency of the microorganisms; as a result, activation of the layer silicate can be influenced in a positive manner.

It has been found that some naturally occurring bentonite/attapulgite clay mixtures already contain low concentrations of T. thiooxidans and T. ferrooxidans. As a rule, however, it is preferable to add the bacteria to the layer silicate. This can take place, for example, by spraying the layer silicate with a concentrated bacterial culture or by mixing with an inoculant material that has a high concentration of bacteria. The following are suitable, in particular, as the inoculant material: a sample of the layer silicate, which is to be activated or which, as the case may be, has already been bacterially activated, or a bacterial substrate (such as sulfur or pyrites) with a concentration of 10^2 - 10^{10} bacteria/g of inoculant material, or mixtures thereof.

In accordance with one ~~form of~~ embodiment in accordance with the invention, a nutrient substrate in the form of sulfur, pyrites and/or a nutrient salt solution is added to the layer silicate for better growth of the microorganisms. The addition of ~~S~~ sulfur is required, in particular, when use is to be made of purely sulfur-oxidizing microorganisms and the layer silicate naturally contains no source of energy (sulfur source or iron source, respectively) that is capable of being utilized by the microorganism in question.

The treatment of the layer silicate with the microorganisms is carried out under conditions that are favorable for the microorganism (or microorganisms) in question. The technical expert will be familiar with these conditions on the basis of the relevant prior art.

Thus, for example, one has to ensure that the microorganisms receive an adequate supply of nutrient substances (e.g. N, K, Ca, Mg, P), vitamins, metabolic substrates, gases (e.g. oxygen, carbon dioxide). If the material that is to be activated naturally contains too little of the substances that are required by the microorganisms that are used in each case, then these can be added to the material.

In the case of using T. ferrooxidans and T. thiooxidans, nutrient salts and/or energy-supplying substrates (e.g. sulfur, pyrites) can be added to the material that is to be activated. Since the designated bacteria are, of necessity, chemolithoautotrophic organisms, these cannot be stimulated via an addition of organic substrates, vitamins or nutrient substances. In some cases, an excessive supply of nutrient salts, in particular, ~~had~~ have a negative influence on the activity of the

microorganisms.

In the case of utilizing Aspergillus niger, ~~use can be made, for example, of~~ glucose, sucrose or molasses ~~as~~ can be added to the substrate.

An adequate water content of the medium or, as the case may be, the layer silicate, and maintenance of a suitable temperature have to be borne in mind as well. Thus, for example, temperatures of approximately 20 to approximately 35°C and, especially, approximately 30°C, and a water content of more than approximately 15% by weight and, in particular, approximately 60 to 70% by weight based on the layer silicate are preferred when using T. ferrooxidans, T. thiooxidans or A. niger. Aqueous suspensions can also be used.

In order to control the water content when carrying out activation in the open air, it can be necessary to guard against intensive irrigation via rainfall (e.g. by storing under a roof, or by applying air-permeable agricultural foils) or to irrigate artificially in the case of dry weather.

The optimum duration of the activation process in individual cases is dependent on the microorganisms that are used and on the nature of the layer silicate that is used and on the ambient conditions, and it can be ascertained with ease by the technical expert via empirical trials on the basis of the decolorizing activity of the layer silicates that have undergone treatment. In general, microbial activation of the layer silicate is carried out over a period of 1 to 150 days. In some cases, however, it can be advantageous to carry out microbial activation over a longer period of time, e.g. for approximately one year. The duration of the activation process can frequently be shortened by carrying out mechanical size reduction of the pieces of the layer silicate after e.g. one week in order to generate new surfaces for bacterial colonization.

In accordance with one form of embodiment, the process in accordance with the invention comprises the following steps: fresh raw clay is broken up into pieces of the order of approximately 2 cm in size; as a result, a large surface area is generated that is accessible to the microorganisms and the air. The clay is then mixed or kneaded with 5-20% of inoculant clay with a high concentration of bacteria and, as a result, colonization with microorganisms is

accelerated. Piles or stacks are formed that are approximately 10-50 cm high. Excessively high heaping up or compaction would prevent effective aeration. The temperature and water content of the clay are checked and kept as constant as possible during microbial activation. Regular and adequate mixing together and aeration of the clay can take place, for example, via a rotary hoe every 1-8 days. The reduction of the pH value can be measured after drying the clay, or directly via a soil pH meter. Part of the activated clay is used as the inoculant clay after the desired degree of activation has been reached (generally between pH 2 and pH 4). The remainder is dried and ground, whereby the microorganisms that are contained in the clay are also killed off.

The use of microorganisms for the activation of layer silicates is also the subject of the invention.

An especially advantageous feature of the process in accordance with the invention is that one does not have to work with corrosive acids that cause intense burns and endanger natural water systems. Thus it ~~[the process]~~ is superior to the prior art from the standpoints of operational safety and environmental protection. Since only very cheap raw materials, such as pyrites (which ~~is~~ are optionally already present in the layer silicate), sulfur and water, are used for microbial activation, the process in accordance with the invention is superior from the economic standpoint as well. Thus the pyrites does not need to be ~~purified~~ removed completely, ~~in the form of a hard accompanying mineral [translator: the meaning of this sentence is not completely clear to me].~~ being a hard accompanying material

It has been found that the pH value or, as the case may be, the quantity of acid that is set free by the microorganisms does not correlate strictly with the activity of the layer silicate that is to be treated. This suggests that microbial activation in accordance with the invention differs from purely acidic activation, and that further metabolic products are involved.

Free iron ions, which are present in the activated layer silicate and which can interfere with the decolorization of oil, are complexed by the Fe-chelating materials that are produced by the microorganisms that are used. In addition, many of the microbial organic acids complex multivalent cations, such as Al^{3+} or Ca^{2+}, and, as a result, these are removed from the equilibrium and activation of the layer silicate ~~is favored.~~

In addition, free phosphate is incorporated into organic compounds via the microorganisms and these organic compounds and the microorganisms adhere firmly to the layer silicate so that phosphate contamination is reduced in the oil that is to be decolorized.

In addition, interfering cations can be fixed (so-called "bio-accumulation") via absorption into the microorganisms. The accumulation of Cd^{2+}, Co^{2+}, Cu^{2+}, Cr^{3+}, Fe^{3+} and Ni^{2+} has been demonstrated in the case of thio-bacteria, and the accumulation of radionuclides, Co^{2+}, Cu^{2+} and Zn^{2+} has been demonstrated in the case of A. niger.

↓ ok

The surface of the mineral is also rendered [more] hydrophobic by the microorganisms. The increased hydrophobicity of the surface of the layer silicate can lead to better wetting of the particles of fuller's earth by the oils that are to be decolorized.

Additional advantages can be traced back to uniform, in situ activation by the microorganisms, and to the gradual release of acids or, as the case may be, metabolic products. Since the microorganisms that are preferably used, such as T. ferrooxidans and T. thiooxidans, no longer grow at excessively low pH values (e.g. less than 1.5), an excessively high residual acid concentration, which is disadvantageous for the decolorization of oils, can also be avoided in the activated layer silicate. As soon as the pH value has declined too much, the microorganisms terminate their growth and the production of acid. The microorganisms thus act like an internal regulating system for the activation of the layer silicate. Local pH peaks, which arise with ease in the case of an external addition of acid, can also be avoided in this way.

The degradation of pyrites, which is contained in the raw clay and which is utilized by T. ferrooxidans as a nutrient substrate during activation, can be advantageous in some applications of the activated layer silicates since pyrites exhibits an abrasive action during the grinding of the fuller's earth.

The examples of embodiments that follow below will demonstrate the invention and the advantages relative to the prior art. However, the invention is not limited to the examples below.

Examples

Examples 1 - 6

Freshly ~~degraded~~ mined attapulgite (palygorskite)/bentonite clay with a solids content of 44% was used as the starting material for comparison Examples 1 and 2 and for Examples 3 - 6. According to x-ray phase analysis and chemical composition [tests] ok, this clay comprises 55% palygorskite, 35% Ca montmorillonite, 5% quartz, 3% calcite and 1.5% pyrites. The clay was mechanically reduced to a grain size of approximately 2 cm. This clay, which had been treated in that way, is designated raw clay A in the following sections.

Example 1 (comparison)

A sample of ~~the~~ raw clay A was dried at 80°C to give a water content of 15% and ground to give a sieve residue (64 µm) of 25%; it was then dried at 110°C to give a water content of 8%. After suspending 8 parts of the sample in 100 parts of water, the pH value of the sample was measured by means of a pH measurement electrode.

Decolorization experiments were carried out using rape-seed oil (100 g of oil; 0.75 g of sample; p = 30 mbar; T = 110°C; t = 30 minutes) and soy oil (100 g of oil; 0.50 g of sample; p = 30 mbar; T = 100°C; t = 30 minutes) in order to ascertain the activity of the sample for decolorizing vegetable oil. The decolorizing activity was assessed on the basis of red values, which were ascertained by means of a Lovibond ~~colorimeter~~ colorimeter, and on the basis of the spectrophotometrically measured chlorophyll concentrations. In both cases, smaller values signify higher decolorizing activity. The results are indicated in Table I (decolorization of rape-seed oil) and Table II (decolorization of soy oil); in every case, the numerical data are average values from three experiments.

Example 2 (comparison)

340 g of raw clay A were intensively kneaded for 5 minutes with 50 ml of water and 3 g of

concentrated sulfuric acid. The product was then dried and ground as in comparison Example 1. The measurement of the pH value and that of the decolorizing activity took place as in comparison Example 1. The results are indicated in Tables I and II.

Example 3

340 g of raw clay A were mixed with 110 ml of water and homogenized with the help of a sterile wooden spatula. Incubation then took place in a climatic chamber at a constant 30°C and 100% atmospheric humidity. Homogenization of the sample was repeated once per week. Each week, a small aliquot portion was taken out and the pH value was measured as described in comparison Example 1.

When no further change in the pH value could be detected (68 days), the product was dried and ground as in comparison Example 1. The measurement of the pH value and that of the decolorizing activity for rape-seed oil took place as in comparison Example 1. The results are indicated in Table I.

Example 4

340 g of raw clay A were incubated as described in Example 3. After 42 days, 45 g of this material were taken out and mixed with 340 g of fresh raw clay A and 110 ml of water and homogenized with the help of a sterile wooden spatula. Incubation then took place in a climatic chamber at a constant 30°C and 100% atmospheric humidity. Homogenization of the sample was repeated once per week. Each week, a small aliquot portion was taken and the pH value was measured as described in comparison Example 1.

When no further change in the pH value could be detected (21 days), the product was dried and ground as in comparison Example 1. The measurement of the pH value and that of the decolorizing activity for rape-seed oil took place as in comparison Example 1. The results are indicated in Table I.

Example 5

1 ml of a suspension of bacteria (Thiobacillus ferrooxidans; DSMZ strain 11477) and 7.0 g of pyrites (particle size < 64 µm) were added to 100 ml of a nutrient medium comprising 2.00 g/l of $(NH_4)_2SO_4$, 0.50 g/l of K_2HPO_4, 0.50 g/l of $MgSO_4.7H_2O$, 0.10 g/l of KCl and 0.01 g/l of $Ca(NO_3)_2$, whereby this nutrient medium had been adjusted to a pH value of 2 using sulfuric acid. A stream of air was ~~led~~ passed through this mixture ~~for sufficiently long~~ at 30°C until the pH value of the solution had fallen to 1.75. The pyrites ~~was~~ were separated from the solution by means of centrifugation at 1,500 g (5 minutes) and then suspended in 100 ml of water and centrifuged again. The bacterial cells were harvested from the combined centrifuged liquids by centrifugation at 8,000 g (15 minutes) and then they were suspended in 110 ml of water.

340 g of raw clay were treated in an autoclave under standard conditions (T = 120°C; p = 2 bar; t = 30 minutes) in order to kill off the microorganisms that were contained in the raw clay. The raw clay was then mixed with 110 ml of bacterial suspension and homogenized with the help of a sterile wooden spatula; incubation then took place in a climatic chamber at a constant 30°C and 100% atmospheric humidity. Homogenization of the sample was repeated once per week. Each week, a small aliquot portion was taken out and the pH value was measured as described in comparison Example 1. When no further change in the pH value could be detected (56 days), the product was dried and ground as in comparison Example 1. The measurement of the pH value and that of the decolorizing activity for rape-seed oil took place as in comparison Example 1. The results are indicated in Table I.

Example 6

Spores of Aspergillus niger (DSMZ strain 823) were added to 110 ml of a sterile nutrient medium comprising 1.60 g/l of NH_4NO_3, 0.30 g/l of K_2HPO_4, 0.20 g/l of $MgSO_4.7H_2O$, and 6.50 g of glucose, whereby this nutrient medium had been adjusted to a pH value of 4.0 using sulfuric acid. A stream of air was ~~led~~ passed through this mixture ~~for sufficiently long~~ at 30°C until the pH value of the solution had fallen to 3.0.

340 g of raw clay were treated in an autoclave under standard conditions (T = 120°C; p = 2 bar; t = 30 minutes) in order to kill off the microorganisms that were contained in the raw clay. The raw clay was then mixed with the fungal suspension and homogenized with the help of a sterile wooden spatula; incubation then took place in a climatic chamber at a constant 30°C and 100% atmospheric humidity. Homogenization of the sample was repeated daily. Each week, a small aliquot portion was taken out and the pH value was measured as described in comparison Example 1. When no further change in the pH value could be detected (21 days), the product was dried and ground as in comparison Example 1. The measurement of the pH value and that of the decolorizing activity for soy oil took place as in comparison Example 1. The results are indicated in Table II.

Examples 7 - 11

South American bentonite with a solids content of 60% was used as the starting material for comparison Examples 7 - 9 and for Examples 10 - 11. According to x-ray phase analysis and chemical composition tests, this clay comprises 90% disordered smectite/illite mixed layer minerals, 2% quartz, 2% calcite and 6% feldspar. The clay was mechanically reduced to a grain size of approximately 2 cm. This clay, which had been treated in that way, ~~is~~ designated "raw clay B" in the following sections.

Example 7 (comparison)

A sample of the raw clay B was dried at 80°C to give a water content of 15% and ground to give a sieve residue (64 µm) of 25%; it was then dried at 100°C to give a water content of 8%. After suspending 8 parts of the sample in 100 parts of water, the pH value of the sample was measured by means of a pH measurement electrode.

Decolorization experiments were carried out using rape-seed oil (100 g of oil; 0.75 g of sample; p = 30 mbar; T = 110°C; t = 30 minutes) and soy oil (100 g of oil; 0.50 g of sample; p = 30 mbar; T = 100°C; t = 30 minutes) in order to ascertain the activity of the sample for decolorizing vegetable oil. The decolorizing activity was assessed on the basis of red values, which were

ascertained by means of a Lovibond color meter, and on the basis of the spectrophotometrically measured chlorophyll concentrations. In both cases, smaller values signify higher decolorizing activity. The results are indicated in Table I (decolorization of rape-seed oil) and Table II (decolorization of soy oil); in every case, the numerical data are average values from three experiments.

Example 8 (comparison)

250 g of raw clay B were intensively kneaded for 5 minutes with 120 ml of water and 3 g of concentrated sulfuric acid. The product was then dried and ground as in comparison Example 7. The measurement of the pH value and that of the decolorizing activity for soy oil and rape-seed oil took place as in comparison Example 7. The results are indicated in Tables I and II.

Example 9 (comparison)

250 g of raw clay B were mixed with 125 ml of water and homogenized with the help of a sterile wooden spatula. Incubation then took place in a climatic chamber at a constant 30°C and 100% atmospheric humidity. Homogenization of the sample was repeated once per week. Each week, a small aliquot portion was taken out and the pH value was measured as described in comparison Example 7.

After 68 days, the product was dried and ground as in comparison Example 7. The measurement of the pH value and that of the decolorizing activity for soy oil and rape-seed oil took place as in comparison Example 7. The results are indicated in Tables I and II.

Example 10

340 g of raw clay A were incubated as described in Example 3.

After 42 days, 46 g of this material were taken out and mixed with 250 g of raw clay B, 7 g of pyrites and 125 ml of water and homogenized with the help of a sterile wooden spatula.

Incubation then took place in a climatic chamber at a constant 30°C and 100% atmospheric humidity. Homogenization of the sample was repeated once per week. Each week, a small aliquot portion was taken and the pH value was measured as described in comparison Example 7.

When no further change in the pH value could be detected (42 days), the product was dried and ground as in comparison Example 7. The measurement of the pH value and that of the decolorizing activity for soy oil and rape-seed oil took place as in comparison Example 7. The results are indicated in Tables I and II.

Example 11

340 g of raw clay A were incubated as described in Example 3.

After 42 days, 46 g of this material were taken out and mixed with 250 g of raw clay B, 7 g of sulfur and 125 ml of water and homogenized with the help of a sterile wooden spatula. Incubation then took place in a climatic chamber at a constant 30°C and 100% atmospheric humidity. Homogenization of the sample was repeated once per week. Each week, a small aliquot portion was taken and the pH value was measured as described in comparison Example 7.

When no further change in the pH value could be detected (56 days), the product was dried and ground as in comparison Example 7. The measurement of the pH value and that of the decolorizing activity took place as in comparison Example 7. The results are indicated in Tables I and II.

Table I: Decolorization of rape-seed oil

	pH	Red value	Chlorophyll A [ppb]	Time [d]	Brief description
Comparison example 1	6.9	5.5	650	0	raw clay A
Comparison example 2	2.8	4.4	300	0	raw clay A + sulfuric acid
Example 3	3.4	4.2	225	68	raw clay A incubated
Example 4	3.4	4.1	220	21	raw clay A + inoculant clay
Example 5	3.4	4.3	240	56	raw clay A inoculated with DSMZ strain
Comparison Example 7	8.4	7.8	800	0	raw clay B
Comparison Example 8	2.3	4.6	310	0	raw clay B + sulfuric acid
Comparison Example 9	8.2	7.8	790	68	raw clay B incubated
Example 10	2.6	4.2	220	42	raw clay B + pyrites + inoculant clay
Example 11	2.8	4.4	290	56	raw clay B + sulfur + inoculant clay

Table II: Decolorization of soy oil

TABLE II, Decolorization of Soybean Oil

	pH	Red value	Chlorophyll A [ppb]	Time [d]	Brief description
Comparison Example 1	**6.9**	**6.4**	**290**	**0**	**raw clay A**
Comparison Example 2	**2.8**	**6.7**	**180**	**0**	**raw clay A + sulfuric acid**
Example 6	**3.4**	**6.0**	**170**	**21**	**raw clay A inoculated with A. niger**
Comparison Example 7	**8.4**	**14.0**	**680**	**0**	**raw clay B**
Comparison Example 8	**2.3**	**9.2**	**170**	**0**	**raw clay B + sulfuric acid**
Comparison Example 9	**8.2**	**13.8**	**690**	**68**	**raw clay B incubated**
Example 10	**2.6**	**6.2**	**150**	**42**	**raw clay B + pyrites + inoculant clay**
Example 11	**2.8**	**6.5**	**170**	**56**	**raw clay B + sulfur + inoculant clay**

As can be seen from Table I, it was possible to induce the ~~wild~~ natural strain populations of T. ferrooxidans and T. thiooxidans, which ~~are~~ were present in raw clay A, to activate the layer silicate by means of suitable conditions. The activated fuller's earth that was obtained exhibited good results for the decolorization of rape-seed oil and surpassed both raw clay A (comparison Example 1) and a fuller's earth (comparison Example 2), which was prepared in accordance with the prior art by activation with concentrated sulfuric acid, in terms of the red values and the removal of chlorophyll.

As Example 4 shows, the duration of activation using raw clay A can be drastically shortened by mixing it with inoculant clay, which already contains large wild strain populations of T. ferrooxidans and T. thiooxidans, with equally good decolorizing activity for rape-seed oil.

A further addition of a nutrient ~~salt~~ solution to the raw clay samples in Examples 3 and 4 did not lead to increased activity of the bacteria in the first 30 days. This can be traced back to the feature that the ~~wild~~ natural strain populations of T. ferrooxidans and T. thiooxidans, which ~~are~~ were present in raw clay A, had become adapted to very low quantities of nutrient salt over a period of many generations.

Example 5 shows that, in addition to ~~wild~~ natural strains, cultivated strains of T. ferrooxidans are also suitable for the activation of the layer silicate in raw clay A. The longer duration of the activation process in comparison to Example 4 can be traced back to the feature that the strains, which have become adapted to higher nutrient salt concentrations, first have to become adapted to the lower concentrations in raw clay A.

As Example 6 documents, it was possible to undertake activation of the layer silicates, which were contained in raw clay A, by means of the Aspergillus niger fungus. Glucose as the nutrient source had to be added to the raw clay in this case. It can be seen from Table II that, relative to comparison Example 1, Example 6 shows considerably better removal of chlorophyll and a better red value for the decolorization of soy oil. By contrast, treatment of raw clay A with sulfuric acid in accordance with the prior art (comparison Example 2) results in almost equally good absorption of chlorophyll but a worsening of the red value, whereby this can be traced back to the

low pH value of the adsorption agent and to the high proportion of residual acid that is associated therewith.

Examples 10 and 11 show that, in addition to pyrites-containing attapulgite earths, other layer silicates are likewise capable of being activated via the use of microorganisms.

As far as the removal of red components and, in particular, chlorophyll is considered, comparison Example 7 shows very bad results for the decolorization of both rape-seed oil and soy oil. According to comparison Example 8, a distinct improvement in decolorization activity is possible via a treatment with sulfuric acid that corresponds to the prior art (see Tables I and II).

If raw clay B is merely incubated (comparison Example 9), then no improvement in decolorizing activity occurs. This can be traced back to the deficiency in energy-supplying accompanying substances (such as e.g. pyrites) in raw clay B and the absence, which is related thereto, of microorganisms (e.g. T. ferrooxidans) that utilize these accompanying substances.

Example 10 shows that activation of the layer silicate in raw clay B can be achieved by an addition of pyrites as the supplier of energy together with inoculant populations of T. ferrooxidans and T. thiooxidans from raw clay A and subsequent incubation. In comparison to raw clay B (comparison Example 7) and raw clay B that had been activated in accordance with the prior art (comparison Example 8), a distinct improvement in decolorizing action is found both in rape-seed oil (Table I) and in soy oil (Table II). The duration of the activation process has been prolonged relative to ~~e.g.~~ Example 4. It is probable that the bacteria, which have become adapted to the conditions in raw clay A, first have to become adapted to the ambient conditions that prevail in raw clay B.

It can be seen from Example 11, that activation of the layer silicate in raw clay B ~~is also possible~~ can also occur by supplying elemental sulfur, followed by inoculation with incubated raw clay A and subsequent incubation. The duration of activation is further prolonged relative to Example 10 because the wild strain populations of T. thiooxidans from raw clay A have to become adapted not only to the changed conditions in raw clay B, but also to the non-adapted energy source. Relative to

comparison Examples 7 and 8, the layer silicate that was activated in accordance with Example 11 exhibits improved activity levels for decolorizing rape-seed oil and soy oil. In comparison to Example 10, lower decolorizing activity was found in the two oils that were investigated; in contrast to this, Example 11 offers the possibility of activation without ~~an~~ the addition of pyrites.

Patent claims

1. Process for the activation of layer silicates, whereby microorganisms are used for activation.

2. Process in accordance with Claim 1, characterized by the feature that a smectitic clay mineral is used as the layer silicate.

3. Process in accordance with Claim 1 or 2, characterized by the feature that a montmorillonite-containing clay, especially bentonite, is used as the layer silicate.

4. Process in accordance with one of the above claims, characterized by the feature that a palygorskite clay or mixtures comprising palygorskite and bentonite are used as the layer silicate.

5. Process in accordance with one of the above claims, characterized by the feature that acid-producing microorganisms are used as the microorganisms.

6. Process in accordance with one of the above claims, characterized by the feature that sulfur-oxidizing bacteria and/or iron-oxidizing bacteria, especially Thiobacillus ferrooxidans and/or Thiobacillus thiooxidans, are used as the microorganisms.

7. Process in accordance with one of the above claims, characterized by the feature that microorganisms that produce citric acid, especially Aspergillus niger, are used as the microorganisms.

8. Process in accordance with one of the above claims, characterized by the feature that the microorganisms are wild type strains, which occur in the layer silicate, or cultivated strains.

9. Process in accordance with one of the above claims, characterized by the feature that the clay is first broken up into pieces with a size of approximately 0.5 cm to approximately 5 cm, especially approximately 2 cm.

10. Process in accordance with one of the above claims, characterized by the feature that the layer silicate is mixed with an inoculant material that has a population of 10^2 to 10^{10} bacteria/g of inoculant material.

11. Process in accordance with one of the above claims, characterized by the feature that sulfur, pyrites, glucose, molasses and/or a nutrient salt solution for the microorganisms is added to the layer silicate.

12. Process in accordance with one of the above claims, characterized by the feature that the treatment with the microorganisms is carried out under growth conditions that are favorable for them, especially at approximately 20 to 35°C and with a water content of more than approximately 15% by weight based on the layer silicate.

13. Process in accordance with one of the above claims, characterized by the feature that the clay is mixed thoroughly and aerated several times during activation.

14. Process in accordance with one of the above claims, characterized by the feature that microbial activation is carried out for 1 to approximately 365 days.

15. Activated layer silicates, obtainable in accordance with one of the above claims.

16. Process for decolorizing oils, fats or waxes that comprises contacting the oil with fuller's earth that is obtainable via a process in accordance one of the above Claims 1 through 14.

17. Use of microorganisms for the activation of layer silicates.

ABSTRACT
~~SUMMARY~~

A process ~~is specified~~ for the microbial activation of layer silicates.

W/Aw

Patent claims

We claim

1. Process for the activation of layer silicates, whereby acid-producing microorganisms are used for activation.

2. Process in accordance with Claim 1, characterized by the feature that a smectitic clay mineral is used as the layer silicate.

3. Process in accordance with Claim 1 or 2, characterized by the feature that a montmorillonite-containing clay, especially bentonite, is used as the layer silicate.

4. Process in accordance with one of the above claims, characterized by the feature that a palygorskite clay or mixtures comprising palygorskite and bentonite are used as the layer silicate.

5. Process in accordance with one of the above claims, characterized by the feature that sulfur-oxidizing bacteria and/or iron-oxidizing bacteria, especially Thiobacillus ferrooxidans and/or Thiobacillus thiooxidans, are used as the microorganisms.

6. Process in accordance with one of the above claims, characterized by the feature that microorganisms that produce citric acid, especially Aspergillus niger, are used as the microorganisms.

7. Process in accordance with one of the above claims, characterized by the feature that the microorganisms are wild type strains, which occur in the layer silicate, or cultivated strains.

8. Process in accordance with one of the above claims, characterized by the feature that the clay is first broken up into pieces with a size of approximately 0.5 cm to approximately 5 cm,

especially approximately 2 cm.

9. Process in accordance with one of the above claims, characterized by the feature that the layer silicate is mixed with an inoculant material that has a population of 10^2 to 10^{10} bacteria/g of inoculant material.

10. Process in accordance with one of the above claims, characterized by the feature that sulfur, pyrites, glucose, molasses and/or a nutrient salt solution for the microorganisms is added to the layer silicate.

11. Process in accordance with one of the above claims, characterized by the feature that the treatment with the microorganisms is carried out under growth conditions that are favorable for them, especially at approximately 20 to 35°C and with a water content of more than approximately 15% by weight based on the layer silicate.

12. Process in accordance with one of the above claims, characterized by the feature that the clay is mixed thoroughly and aerated several times during activation.

13. Process in accordance with one of the above claims, characterized by the feature that microbial activation is carried out for 1 to approximately 365 days.

14. Activated layer silicates, obtainable in accordance with one of the above claims.

15. Process for decolorizing oils, fats or waxes that comprises contacting the oil with fuller's earth that is obtainable via a process in accordance one of the above Claims 1 through 14.

16. Use of acid-producing microorganisms for the preparation of fuller's earths for the treatment of oils, fats or waxes.

Patent application

Microbial activation of layer silicates

Specification

The invention pertains to a process for the activation of layer silicates with use being made of microorganisms.

US 1,492,184 describes the activation of raw clay with maximally 10% by weight of concentrated acid. It is preferable that a pre-dried and ground raw clay be impregnated in this regard. Montmorillonite, bauxite, willonite, pyrophyllite, kaolinite and fuller's earth are designated as examples of "clays".

US 1,752,721 describes a process for the treatment of "earthy materials" in order to increase their adsorption properties; accordingly, a clay material is mixed with solid oxalic acid and then it is heated, in the absence of added water, in order to bring about a reaction between the oxalic acid and the clay mineral. The clay mineral is treated with approximately 1 to 5% of oxalic acid in this connection.

DE-C-304706 describes a process for increasing the decolorizing power of fuller's earth. In this process, the raw fuller's earth is stirred with the acid to give a doughy mass, and then it is dried.

A process for the treatment of clay is known from US 4,847,226 in which the clay is extruded, ground and then added to an aqueous solution of an acid in order to produce a suspension; the suspension is heated, and then the acid-treated clay is separated, washed, filtered off and dried. The intended purpose of the treatment is to improve the ability of the clay to filter out impurities from liquids. In particular, oil-soluble dyes are removed from oils.

The objective of the invention is to provide a process for the activation of layer silicates without the addition of corrosive acids that can cause intense burns and that endanger natural water systems,

whereby this process is superior to the prior art from the standpoints of operational safety and environmental protection and also from an economic point of view.

Surprisingly, it has now been found that the activation of layer silicates can take place via the use of microorganisms without any addition of an acid or, as the case may be, a solution of an acid.

The use of acid-producing microorganisms for leaching out residues from low grade copper ores is already known in the prior art. In addition, the growth of such microorganisms on ores, such as pyrites, is exploited in order to assist flotation. A review of these and further applications of the treatment and processing of ores and for metal recovery is to be found in the publication by C.L. Brierley: Bacteria as aids in mining; Spetrum der Wissenschaft: Industrielle Mikrobiologie, 60 (1989).

The bacterial oxidation of elemental sulfur is exploited in agriculture in order to make sulfate available to plants and to make phosphate and micro-nutrient substances available as well.

The use of microorganisms for the activation of layer silicates is not known in the prior art.

The term "activation" is understood to mean a process for increasing the decolorizing activity of the layer silicate.

The activated layer silicates in accordance with the invention can be used, in particular, as fuller's earth materials for the treatment of oils, fats or waxes.

Glyceride oils, waxes and fats and mineral oils are passed through one or more adsorptive treatment stages using inorganic adsorbents during their refining. The oil or fat that is to be treated is thereby brought into contact with an inorganic adsorbent at an elevated temperature. The adsorbent filters from the oil substances that are disadvantageous in subsequent processes or for storage, such as e.g. pigments, phospholipids, materials that produce turbidity, metals, free fatty acids, oxidized

compounds, etc. In order to do this, the adsorbent requires adsorptive properties, in order to permit the removal of phospholipids or chlorophyll materials, and catalytic properties for degrading dyes or peroxide compounds that are contained in the oil.

Because of their advantageous properties, especially their high specific surface area, sorption capacity and ion exchange capacity, the activated layer silicates that are prepared in accordance with the invention can also find use in other sectors.

The layer silicates that are listed in Ullmann's Encyklopädie der technischen Chemie ["Encyclopedia of industrial Chemistry"], Volume 21, pages 370-375 (1982) are included among the layer silicates that are usable in the process in accordance with the invention. In particular, use can be made of natural and synthetic clay minerals that are capable of being activated, such as the smectites - including montmorillonite, beidellite, nontronite, wolchonskoite, stevensite, hectorite, swinefordite, saponite and sauconite - along with vermiculites, illites, mixed layer minerals, palygorskite (attapulgite) and sepiolite. The latter two materials are also designated as hormites. The clay minerals can be present in their H form, their alkali metal form or their alkaline earth form.

In accordance with a preferred form of embodiment in accordance with the invention, the layer silicate is a three layer silicate, e.g. a naturally occurring smectitic clay, especially a bentonite clay or a palygorskite clay or mixtures thereof.

Palygorskite clays comprise attapulgite clays that are also known as attalpulgus clays, or Georgia fuller's earths. As a rule, these clays consist primarily of the mineral attapulgite, i.e. a crystalline, hydrated magnesium aluminum silicate, but they can also contain considerable quantities of other minerals, such as e.g. bentonite (montmorillonite), calcium carbonate, quartz and feldspar and, in many cases, sepiolite. The preferred clays contain at least 10% by weight, and up to 90% by weight, of attapulgite and, preferably, up to 20 to 60% by weight thereof.

Non-calcined, naturally occurring mixtures of palygorskite clay and calcium bentonite clay are especially preferred. Such natural mixtures can contain pyrites that can serve as a substrate for sulfur-oxidizing bacteria and iron-oxidizing bacteria such as Thiobacillus ferrooxidans.

An attapulgite/bentonite mixture of clays is used in accordance with an especially preferred form of embodiment.

The microorganisms that are used for activation in accordance with the invention are bacteria, archaebacteria or fungi e.g. of the Aspergillus, Acidianus, Acidimicrobium, Acidiphilium, Acidobacterium, Acidocella, Alicyclobacillus, Leptospirillium, Metallosphaera, Picrophylus, Sarcina, Stygiolobus, Sulfobacillus, Sulfolobus, Thermoplasma, Thiobacillus and Thiomonas strains. In addition to the acid-producing bacteria that are preferred here - especially the so-called sulfuric acid bacteria - use can also be made of nitric acid bacteria and acetic acid bacteria as well as microorganisms that produce oxalic acid, citric acid, gluconic acid or other organic acids.

The use of bacteria that oxidize pyrites is especially advantageous when the layer silicate that is used already contains pyrites, so that this nutrient substance for the bacteria does not have to be added. In addition, it has been found that some naturally occurring bentonite/attapulgite clay mixtures already naturally contain Thiobacillus ferrooxidans and Thiobacillus thiooxidans in small quantities, and these can be induced to activate the layer silicate.

The last two types of bacteria that were designated are strongly chemolithoautotrophic, i.e. their growth cannot be stimulated by providing organic materials, such as nutrient substances or vitamins. Both belong to the group of acidophilic bacteria and they prefer pH values around 2 and temperatures of around 30°C.

Use can be made of both natural type strains of the microorganisms and also strains that have been cultivated in the laboratory (e.g. Thiobacillus thiooxidans DSMZ-11478; Aspergillus niger DSMZ-823; see the DSMZ catalog, 1998). Prior cultivation of the microorganisms offers the advantage that

adaptation can be carried out using the conditions that have been selected for the activation of the layer silicate. In addition, the microorganisms can be selected conventionally in terms of advantageous properties (e.g. rapid growth under the conditions that have been selected for activation).

In the case of using aerobic microorganisms (such as, for example, T. thiooxidans, T. ferrooxidans and A. niger), an adequate supply of oxygen has to be ensured during activation of the layer silicate. This can be ensured, for example, by regular mixing in with the layer silicate (every 1 to 7 days) and avoiding excessive compaction. The process of mixing in also encourages uniform distribution, more rapid multiplication and higher metabolic efficiency of the microorganisms; as a result, activation of the layer silicate can be influenced in a positive manner.

It has been found that some naturally occurring bentonite/attapulgite clay mixtures already contain low concentrations of T. thiooxidans and T. ferrooxidans. As a rule, however, it is preferable to add the bacteria to the layer silicate. This can take place, for example, by spraying the layer silicate with a concentrated bacterial culture or by mixing with an inoculant material that has a high concentration of bacteria. The following are suitable, in particular, as the inoculant material: a sample of the layer silicate, which is to be activated or which, as the case may be, has already been bacterially activated, or a bacterial substrate (such as sulfur or pyrites) with a concentration of 10^2 - 10^{10} bacteria/g of inoculant material, or mixtures thereof.

In accordance with one embodiment in accordance with the invention, a nutrient substrate in the form of sulfur, pyrites and/or a nutrient salt solution is added to the layer silicate for better growth of the microorganisms. The addition of sulfur is required, in particular, when use is to be made of purely sulfur-oxidizing microorganisms and the layer silicate naturally contains no source of energy (sulfur source or iron source, respectively) that is capable of being utilized by the microorganism in question.

The treatment of the layer silicate with the microorganisms is carried out under conditions that are favorable for the microorganism (or microorganisms) in question. The technical expert will be familiar with these conditions on the basis of the relevant prior art.

Thus, for example, one has to ensure that the microorganisms receive an adequate supply of nutrient substances (e.g. N, K, Ca, Mg, P), vitamins, metabolic substrates, gases (e.g. oxygen, carbon dioxide). If the material that is to be activated naturally contains too little of the substances that are required by the microorganisms that are used in each case, then these can be added to the material.

In the case of using T. ferrooxidans and T. thiooxidans, nutrient salts and/or energy-supplying substrates (e.g. sulfur, pyrites) can be added to the material that is to be activated. Since the designated bacteria are, of necessity, chemolithoautotrophic organisms, these cannot be stimulated via an addition of organic substrates, vitamins or nutrient substances. In some cases, an excessive supply of nutrient salts, in particular, have a negative influence on the activity of the microorganisms.

In the case of utilizing Aspergillus niger, glucose, sucrose or molasses can be added to the substrate.

An adequate water content of the medium or, as the case may be, the layer silicate, and maintenance of a suitable temperature have to be borne in mind as well. Thus, for example, temperatures of approximately 20 to approximately 35°C and, especially, approximately 30°C, and a water content of more than approximately 15% by weight and, in particular, approximately 60 to 70% by weight based on the layer silicate are preferred when using T. ferrooxidans, T. thiooxidans or A. niger. Aqueous suspensions can also be used.

In order to control the water content when carrying out activation in the open air, it can be necessary to guard against intensive irrigation via rainfall (e.g. by storing under a roof, or by applying air-permeable agricultural foils) or to irrigate artificially in the case of dry weather.

The optimum duration of the activation process in individual cases is dependent on the microorganisms that are used and on the nature of the layer silicate that is used and on the ambient conditions, and it can be ascertained with ease by the technical expert via empirical trials on the basis of the decolorizing activity of the layer silicates that have undergone treatment. In general, microbial activation of the layer silicate is carried out over a period of 1 to 150 days. In some cases, however, it can be advantageous to carry out microbial activation over a longer period of time, e.g. for approximately one year. The duration of the activation process can frequently be shortened by carrying out mechanical size reduction of the pieces of the layer silicate after e.g. one week in order to generate new surfaces for bacterial colonization.

In accordance with one form of embodiment, the process in accordance with the invention comprises the following steps: fresh raw clay is broken up into pieces of the order of approximately 2 cm in size; as a result, a large surface area is generated that is accessible to the microorganisms and the air. The clay is then mixed or kneaded with 5-20% of inoculant clay with a high concentration of bacteria and, as a result, colonization with microorganisms is accelerated. Piles or stacks are formed that are approximately 10-50 cm high. Excessively high heaping up or compaction would prevent effective aeration. The temperature and water content of the clay are checked and kept as constant as possible during microbial activation. Regular and adequate mixing together and aeration of the clay can take place, for example, via a rotary hoe every 1-8 days. The reduction of the pH value can be measured after drying the clay, or directly via a soil pH meter. Part of the activated clay is used as the inoculant clay after the desired degree of activation has been reached (generally between pH 2 and pH 4). The remainder is dried and ground, whereby the microorganisms that are contained in the clay are also killed off.

The use of microorganisms for the activation of layer silicates is also the subject of the invention.

An especially advantageous feature of the process in accordance with the invention is that one does not have to work with corrosive acids that cause intense burns and endanger natural water systems. Thus it is superior to the prior art from the standpoints of operational safety and environmental

protection. Since only very cheap raw materials, such as pyrites (which are optionally already present in the layer silicate), sulfur and water, are used for microbial activation, the process in accordance with the invention is superior from the economic standpoint as well. Thus the pyrites being a hard accompanying material do not need to be removed completely.

It has been found that the pH value or, as the case may be, the quantity of acid that is set free by the microorganisms does not correlate strictly with the activity of the layer silicate that is to be treated. This suggests that microbial activation in accordance with the invention differs from purely acidic activation, and that further metabolic products are involved.

Free iron ions, which are present in the activated layer silicate and which can interfere with the decolorization of oil, are complexed by the Fe-chelating materials that are produced by the microorganisms that are used. In addition, many of the microbial organic acids complex multivalent cations, such as Al^{3+} or Ca^{2+}, and, as a result, these are removed from the equilibrium and activation of the layer silicate.

In addition, free phosphate is incorporated into organic compounds via the microorganisms and these organic compounds and the microorganisms adhere firmly to the layer silicate so that phosphate contamination is reduced in the oil that is to be decolorized.

In addition, interfering cations can be fixed (so-called "bio-accumulation") via absorption into the microorganisms. The accumulation of Cd^{2+}, Co^{2+}, Cu^{2+}, Cr^{3+}, Fe^{3+} and Ni^{2+} has been demonstrated in the case of thio-bacteria, and the accumulation of radionuclides, Co^{2+}, Cu^{2+} and Zn^{2+} has been demonstrated in the case of A. niger.

The surface of the mineral is also rendered more hydrophobic by the microorganisms. The increased hydrophobicity of the surface of the layer silicate can lead to better wetting of the particles of fuller's earth by the oils that are to be decolorized.

Additional advantages can be traced back to uniform, in situ activation by the microorganisms, and to the gradual release of acids or, as the case may be, metabolic products. Since the microorganisms that are preferably used, such as T. ferrooxidans and T. thiooxidans, no longer grow at excessively low pH values (e.g. less than 1.5), an excessively high residual acid concentration, which is disadvantageous for the decolorization of oils, can also be avoided in the activated layer silicate. As soon as the pH value has declined too much, the microorganisms terminate their growth and the production of acid. The microorganisms thus act like an internal regulating system for the activation of the layer silicate. Local pH peaks, which arise with ease in the case of an external addition of acid, can also be avoided in this way.

The degradation of pyrites, which is contained in the raw clay and which is utilized by T. ferrooxidans as a nutrient substrate during activation, can be advantageous in some applications of the activated layer silicates since pyrites exhibits an abrasive action during the grinding of the fuller's earth.

The examples of embodiments that follow below will demonstrate the invention and the advantages relative to the prior art. However, the invention is not limited to the examples below.

Examples

Examples 1 - 6

Freshly mined attapulgite (palygorskite)/bentonite clay with a solids content of 44% was used as the starting material for comparison Examples 1 and 2 and for Examples 3 - 6. According to x-ray phase analysis and chemical composition tests, this clay comprises 55% palygorskite, 35% Ca montmorillonite, 5% quartz, 3% calcite and 1.5% pyrites. The clay was mechanically reduced to a grain size of approximately 2 cm. This clay, which had been treated in that way, is designated raw clay A in the following sections.

Example 1 (comparison)

A sample of raw clay A was dried at 80°C to give a water content of 15% and ground to give a sieve residue (64 ìm) of 25%; it was then dried at 110°C to give a water content of 8%. After suspending 8 parts of the sample in 100 parts of water, the pH value of the sample was measured by means of a pH measurement electrode.

Decolorization experiments were carried out using rape-seed oil (100 g of oil; 0.75 g of sample; p = 30 mbar; T = 110°C; t = 30 minutes) and soy oil (100 g of oil; 0.50 g of sample; p = 30 mbar; T = 100°C; t = 30 minutes) in order to ascertain the activity of the sample for decolorizing vegetable oil. The decolorizing activity was assessed on the basis of red values, which were ascertained by means of a Lovibond colorimeter, and on the basis of the spectrophotometrically measured chlorophyll concentrations. In both cases, smaller values signify higher decolorizing activity. The results are indicated in Table I (decolorization of rape-seed oil) and Table II (decolorization of soy oil); in every case, the numerical data are average values from three experiments.

Example 2 (comparison)

340 g of raw clay A were intensively kneaded for 5 minutes with 50 ml of water and 3 g of concentrated sulfuric acid. The product was then dried and ground as in comparison Example 1. The measurement of the pH value and that of the decolorizing activity took place as in comparison Example 1. The results are indicated in Tables I and II.

Example 3

340 g of raw clay A were mixed with 110 ml of water and homogenized with the help of a sterile wooden spatula. Incubation then took place in a climatic chamber at a constant 30°C and 100% atmospheric humidity. Homogenization of the sample was repeated once per week. Each week, a

small aliquot portion was taken out and the pH value was measured as described in comparison Example 1.

When no further change in the pH value could be detected (68 days), the product was dried and ground as in comparison Example 1. The measurement of the pH value and that of the decolorizing activity for rape-seed oil took place as in comparison Example 1. The results are indicated in Table I.

Example 4

340 g of raw clay A were incubated as described in Example 3. After 42 days, 45 g of this material were taken out and mixed with 340 g of fresh raw clay A and 110 ml of water and homogenized with the help of a sterile wooden spatula. Incubation then took place in a climatic chamber at a constant 30°C and 100% atmospheric humidity. Homogenization of the sample was repeated once per week. Each week, a small aliquot portion was taken and the pH value was measured as described in comparison Example 1.

When no further change in the pH value could be detected (21 days), the product was dried and ground as in comparison Example 1. The measurement of the pH value and that of the decolorizing activity for rape-seed oil took place as in comparison Example 1. The results are indicated in Table I.

Example 5

1 ml of a suspension of bacteria (Thiobacillus ferrooxidans; DSMZ strain 11477) and 7.0 g of pyrites (particle size < 64 ìm) were added to 100 ml of a nutrient medium comprising 2.00 g/l of $(NH_4)_2SO_4$, 0.50 g/l of K_2HPO_4, 0.50 g/l of $MgSO_4.7H_2O$, 0.10 g/l of KCl and 0.01 g/l of $Ca(NO_3)_2$, whereby this nutrient medium had been adjusted to a pH value of 2 using sulfuric acid. A stream of air was passed through this mixture at 30°C until the pH value of the solution had fallen to 1.75. The pyrites

were separated from the solution by means of centrifugation at 1,500 g (5 minutes) and then suspended in 100 ml of water and centrifuged again. The bacterial cells were harvested from the combined centrifuged liquids by centrifugation at 8,000 g (15 minutes) and then they were suspended in 110 ml of water.

340 g of raw clay were treated in an autoclave under standard conditions (T = 120°C; p = 2 bar; t = 30 minutes) in order to kill off the microorganisms that were contained in the raw clay. The raw clay was then mixed with 110 ml of bacterial suspension and homogenized with the help of a sterile wooden spatula; incubation then took place in a climatic chamber at a constant 30°C and 100% atmospheric humidity. Homogenization of the sample was repeated once per week. Each week, a small aliquot portion was taken out and the pH value was measured as described in comparison Example 1. When no further change in the pH value could be detected (56 days), the product was dried and ground as in comparison Example 1. The measurement of the pH value and that of the decolorizing activity for rape-seed oil took place as in comparison Example 1. The results are indicated in Table I.

Example 6

Spores of Aspergillus niger (DSMZ strain 823) were added to 110 ml of a sterile nutrient medium comprising 1.60 g/l of NH_4NO_3, 0.30 g/l of K_2HPO_4, 0.20 g/l of $MgSO_4.7H_2O$, and 6.50 g of glucose, whereby this nutrient medium had been adjusted to a pH value of 4.0 using sulfuric acid. A stream of air was passed through this mixture at 30°C until the pH value of the solution had fallen to 3.0.

340 g of raw clay were treated in an autoclave under standard conditions (T = 120°C; p = 2 bar; t = 30 minutes) in order to kill off the microorganisms that were contained in the raw clay. The raw clay was then mixed with the fungal suspension and homogenized with the help of a sterile wooden spatula; incubation then took place in a climatic chamber at a constant 30°C and 100% atmospheric humidity. Homogenization of the sample was repeated daily. Each week, a small aliquot portion was taken out and the pH value was measured as described in comparison Example 1. When no

further change in the pH value could be detected (21 days), the product was dried and ground as in comparison Example 1. The measurement of the pH value and that of the decolorizing activity for soy oil took place as in comparison Example 1. The results are indicated in Table II.

Examples 7 - 11

South American bentonite with a solids content of 60% was used as the starting material for comparison Examples 7 - 9 and for Examples 10 - 11. According to x-ray phase analysis and chemical composition tests, this clay comprises 90% disordered smectite/illite mixed layer minerals, 2% quartz, 2% calcite and 6% feldspar. The clay was mechanically reduced to a grain size of approximately 2 cm. This clay, which had been treated in that way, was designated "raw clay B" in the following sections.

Example 7 (comparison)

A sample of the raw clay B was dried at 80°C to give a water content of 15% and ground to give a sieve residue (64 ìm) of 25%; it was then dried at 100°C to give a water content of 8%. After suspending 8 parts of the sample in 100 parts of water, the pH value of the sample was measured by means of a pH measurement electrode.

Decolorization experiments were carried out using rape-seed oil (100 g of oil; 0.75 g of sample; p = 30 mbar; T = 110°C; t = 30 minutes) and soy oil (100 g of oil; 0.50 g of sample; p = 30 mbar; T = 100°C; t = 30 minutes) in order to ascertain the activity of the sample for decolorizing vegetable oil. The decolorizing activity was assessed on the basis of red values, which were ascertained by means of a Lovibond color meter, and on the basis of the spectrophotometrically measured chlorophyll concentrations. In both cases, smaller values signify higher decolorizing activity. The results are indicated in Table I (decolorization of rape-seed oil) and Table II (decolorization of soy oil); in every case, the numerical data are average values from three experiments.

Example 8 (comparison)

250 g of raw clay B were intensively kneaded for 5 minutes with 120 ml of water and 3 g of concentrated sulfuric acid. The product was then dried and ground as in comparison Example 7. The measurement of the pH value and that of the decolorizing activity for soy oil and rape-seed oil took place as in comparison Example 7. The results are indicated in Tables I and II.

Example 9 (comparison)

250 g of raw clay B were mixed with 125 ml of water and homogenized with the help of a sterile wooden spatula. Incubation then took place in a climatic chamber at a constant 30°C and 100% atmospheric humidity. Homogenization of the sample was repeated once per week. Each week, a small aliquot portion was taken out and the pH value was measured as described in comparison Example 7.

After 68 days, the product was dried and ground as in comparison Example 7. The measurement of the pH value and that of the decolorizing activity for soy oil and rape-seed oil took place as in comparison Example 7. The results are indicated in Tables I and II.

Example 10

340 g of raw clay A were incubated as described in Example 3.

After 42 days, 46 g of this material were taken out and mixed with 250 g of raw clay B, 7 g of pyrites and 125 ml of water and homogenized with the help of a sterile wooden spatula. Incubation then took place in a climatic chamber at a constant 30°C and 100% atmospheric humidity. Homogenization of the sample was repeated once per week. Each week, a small aliquot portion was taken and the pH value was measured as described in comparison Example 7.

When no further change in the pH value could be detected (42 days), the product was dried and ground as in comparison Example 7. The measurement of the pH value and that of the decolorizing activity for soy oil and rape-seed oil took place as in comparison Example 7. The results are indicated in Tables I and II.

Example 11

340 g of raw clay A were incubated as described in Example 3.

After 42 days, 46 g of this material were taken out and mixed with 250 g of raw clay B, 7 g of sulfur and 125 ml of water and homogenized with the help of a sterile wooden spatula. Incubation then took place in a climatic chamber at a constant 30°C and 100% atmospheric humidity. Homogenization of the sample was repeated once per week. Each week, a small aliquot portion was taken and the pH value was measured as described in comparison Example 7.

When no further change in the pH value could be detected (56 days), the product was dried and ground as in comparison Example 7. The measurement of the pH value and that of the decolorizing activity took place as in comparison Example 7. The results are indicated in Tables I and II.

Table I: Decolorization of rape-seed oil

	pH	Red value	Chlorophyll A [ppb]	Time [d]	Brief description
Comparison example 1	6.9	5.5	650	0	raw clay A
Comparison example 2	2.8	4.4	300	0	raw clay A + sulfuric acid
Example 3	3.4	4.2	225	68	raw clay A incubated
Example 4	3.4	4.1	220	21	raw clay A + inoculant clay
Example 5	3.4	4.3	240	56	raw clay A inoculated with DSMZ strain
Comparison Example 7	8.4	7.8	800	0	raw clay B
Comparison Example 8	2.3	4.6	310	0	raw clay B + sulfuric acid
Comparison Example 9	8.2	7.8	790	68	raw clay B incubated
Example 10	2.6	4.2	220	42	raw clay B + pyrites + inoculant clay
Example 11	2.8	4.4	290	56	raw clay B + sulfur +

inoculant clay

Table II: Decolorization of soybean oil

	pH	Red value	Chlorophyll A [ppb]	Time [d]	Brief description
Comparison Example 1	6.9	6.4	290	0	raw clay A
Comparison Example 2	2.8	6.7	180	0	raw clay A + sulfuric acid
Example 6	3.4	6.0	170	21	raw clay A inoculated with A. niger
Comparison Example 7	8.4	14.0	680	0	raw clay B
Comparison Example 8	2.3	9.2	170	0	raw clay B + sulfuric acid
Comparison Example 9	8.2	13.8	690	68	raw clay B incubated
Example 10	2.6	6.2	150	42	raw clay B + pyrites + inoculant clay
Example 11	2.8	6.5	170	56	raw clay B + sulfur + inoculant clay

As can be seen from Table I, it was possible to induce the natural strain populations of T. ferrooxidans and T. thiooxidans, which were present in raw clay A, to activate the layer silicate by means of suitable conditions. The activated fuller's earth that was obtained exhibited good results for the decolorization of rape-seed oil and surpassed both raw clay A (comparison Example 1) and a fuller's earth (comparison Example 2), which was prepared in accordance with the prior art by activation with concentrated sulfuric acid, in terms of the red values and the removal of chlorophyll.

As Example 4 shows, the duration of activation using raw clay A can be drastically shortened by mixing it with inoculant clay, which already contains large wild strain populations of T. ferrooxidans and T. thiooxidans, with equally good decolorizing activity for rape-seed oil.

A further addition of a nutrient solution to the raw clay samples in Examples 3 and 4 did not lead to increased activity of the bacteria in the first 30 days. This can be traced back to the feature that the natural strain populations of T. ferrooxidans and T. thiooxidans, which were present in raw clay A, had become adapted to very low quantities of nutrient salt over a period of many generations.

Example 5 shows that, in addition to natural strains, cultivated strains of T. ferrooxidans are also suitable for the activation of the layer silicate in raw clay A. The longer duration of the activation process in comparison to Example 4 can be traced back to the feature that the strains, which have become adapted to higher nutrient salt concentrations, first have to become adapted to the lower concentrations in raw clay A.

As Example 6 documents, it was possible to undertake activation of the layer silicates, which were contained in raw clay A, by means of the Aspergillus niger fungus. Glucose as the nutrient source had to be added to the raw clay in this case. It can be seen from Table II that, relative to comparison Example 1, Example 6 shows considerably better removal of chlorophyll and a better red value for the decolorization of soy oil. By contrast, treatment of raw clay A with sulfuric acid in accordance with the prior art (comparison Example 2) results in almost equally good absorption of chlorophyll

but a worsening of the red value, whereby this can be traced back to the low pH value of the adsorption agent and to the high proportion of residual acid that is associated therewith.

Examples 10 and 11 show that, in addition to pyrites-containing attapulgite earths, other layer silicates are likewise capable of being activated via the use of microorganisms.

As far as the removal of red components and, in particular, chlorophyll is considered, comparison Example 7 shows very bad results for the decolorization of both rape-seed oil and soy oil. According to comparison Example 8, a distinct improvement in decolorization activity is possible via a treatment with sulfuric acid that corresponds to the prior art (see Tables I and II).

If raw clay B is merely incubated (comparison Example 9), then no improvement in decolorizing activity occurs. This can be traced back to the deficiency in energy-supplying accompanying substances (such as e.g. pyrites) in raw clay B and the absence, which is related thereto, of microorganisms (e.g. T. ferrooxidans) that utilize these accompanying substances.

Example 10 shows that activation of the layer silicate in raw clay B can be achieved by an addition of pyrites as the supplier of energy together with inoculant populations of T. ferrooxidans and T. thiooxidans from raw clay A and subsequent incubation. In comparison to raw clay B (comparison Example 7) and raw clay B that had been activated in accordance with the prior art (comparison Example 8), a distinct improvement in decolorizing action is found both in rape-seed oil (Table I) and in soy oil (Table II). The duration of the activation process has been prolonged relative to Example 4. It is probable that the bacteria, which have become adapted to the conditions in raw clay A, first have to become adapted to the ambient conditions that prevail in raw clay B.

It can be seen from Example 11, that activation of the layer silicate in raw clay B can also occur by supplying elemental sulfur, followed by inoculation with incubated raw clay A and subsequent incubation. The duration of activation is further prolonged relative to Example 10 because the wild strain populations of T. thiooxidans from raw clay A have to become adapted not only to the

changed conditions in raw clay B, but also to the non-adapted energy source. Relative to comparison Examples 7 and 8, the layer silicate that was activated in accordance with Example 11 exhibits improved activity levels for decolorizing rape-seed oil and soy oil. In comparison to Example 10, lower decolorizing activity was found in the two oils that were investigated; in contrast to this, Example 11 offers the possibility of activation without the addition of pyrites.

Patent claims

17. A process for the activation of a layered silicate for treatment of oils, fats and waxes comprising

preparing a layered silicate composition,

activating that layered silicate composition by treating the layered silicate composition with an acid-producing microorganism.

18. The process of Claim 17 wherein the layered silicate comprises a smectite clay.

19. The process of Claim 17 wherein the layered silicate comprises a montmorillonite clay.

20. The process of Claim 19 wherein the montmorillonite clay comprises a bentonite clay.

21. The process of Claim 17 wherein the layered silicate comprises a palygorskite clay.

22. The process of Claim 20 wherein the layered silicate further comprises a palygorskite clay.

23. The process of Claim 17 wherein the acid-producing microorganism comprises a sulfur-oxidizing bacteria.

24. The process of Claim 17 wherein the acid-producing microorganism comprises an iron-oxidizing bacteria.

25. The process of Claim 23 wherein the sulfur-oxidizing bacteria comprises Thiobacillus thiooxidans.

26. The process of Claim 24 wherein the iron-oxidizing bacteria comprises Thiobacillus ferrooxidans.

27. The process of Claim 17 wherein the acid-producing microorganism produces citric acid.

28. The process of Claim 27 wherein the citric acid-producing microorganism comprises Aspergillus niger.

29. The process of Claim 17 further comprising breaking up the layered silicate composition prior to activation into clumps with a size from about 0.5 cm to about 5 cm.

30. The process of Claim 17 further comprising adding the acid-producing microorganisms to an inoculant material prior to activating the layered silicate composition with the microorganisms which have been added to the inoculant material.

31. The process of Claim 30 wherein the population of the microorganisms added to the layered silicate is from about 10^2 to about 10^{10} bacteria/g of the inoculant material.

32. The process of Claim 17 further comprising maintaining the temperature of the layered silicate composition during activation within the range from about 20 to about 35°C.

33. The process of Claim 17 further comprising maintaining the water content of the layered silicate composition during the activating process within a range from about 15 percent by weight to about 70 percent by weight.

34. The process of Claim 30 wherein the inoculant material added to the layered silicate comprises about 5 to about 20 percent of the overall composition after the inoculant material has been added.

35. The process of Claim 17 further comprising mixing and aerating the layered silicate composition while it is being activated with the acid-producing microorganism.

36. The process of Claim 35 wherein the activation process occurs for a period of time from about 1 to about 365 days.

37. The process of Claim 17 further comprising adding nutrients for the microorganisms to the layered silicate composition prior to activation.

38. The process of Claim 37 wherein the nutrients added comprise sulfur-containing products.

39. The process of Claim 17 further comprising adding small quantities of a dilute acid to the layered silicate composition prior to activation with the acid-producing microorganisms.

40. An activated layered silicate prepared by the process of Claim 17.

41. A process for decolorizing oils, fats or waxes comprising contacting the oils, fats or waxes with the activated layered silicate prepared by the process of Claim 17.

ABSTRACT

A process for the microbial activation of layer silicates.

TRANSLATION FROM GERMAN

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Patent application

Microbial activation of layer silicates

Specification

The invention pertains to a process for the activation of layer silicates with use being made of microorganisms.

US 1,492,184 describes the activation of raw clay with maximally 10% by weight of concentrated acid. It is preferable that a pre-dried and ground raw clay be impregnated in this

regard. Montmorillonite, bauxite, willonite, pyrophyllite, kaolinite and fuller's earth are designated as examples of "clays".

US 1,752,721 describes a process for the treatment of "earthy materials" in order to increase their adsorption properties; accordingly, a clay material is mixed with solid oxalic acid and then it is heated, in the absence of added water, in order to bring about a reaction between the oxalic acid and the clay mineral. The clay mineral is treated with approximately 1 to 5% of oxalic acid in this connection.

DE-C-304706 describes a process for increasing the decolorizing power of fuller's earth. In this process, the raw fuller's earth is stirred with the acid to give a doughy mass, and then it is dried.

A process for the treatment of clay is known from US 4,847,226 in which the clay is extruded, ground and then added to an aqueous solution of an acid in order to produce a suspension; the suspension is heated, and then the acid-treated clay is separated, washed, filtered off and dried. The intended purpose of the treatment is to improve the ability of the clay to filter out impurities from liquids. In particular, oil-soluble dyes are removed from oils.

The objective that now forms the basis of the invention is to provide a process for the activation of layer silicates that can be carried out without the addition of corrosive acids that cause intense burns and that endanger natural water systems, whereby this process is superior to the prior art from the standpoints of operational safety and environmental protection and also from the economic point of view.

Surprisingly, it has now been found that the activation of layer silicates can take place via the use of microorganisms without any addition of an acid or, as the case may be, a solution of an acid.

The use of acid-producing microorganisms for leaching out residues from low grade copper ores is already known in the prior art. In addition, the growth of such microorganisms on ores, such as pyrites, is exploited in order to assist flotation. A review of these and further applications of the treatment and processing of ores and for metal recovery is to be found in the publication by

C.L. Brierley: Bacteria as aids in mining; the spectrum of this science: Industrielle Mikrobiologie, 60 (1989).

The bacterial oxidation of elemental sulfur is exploited in agriculture in order to make sulfate available to the plants and to make phosphate and micro-nutrient substances available as well.

The use of microorganisms for the activation of layer silicates is not known in the prior art.

The subject of the invention is a process in accordance with Claim 1. The term "activation" is to be understood to mean a process for increasing the decolorizing activity of the layer silicate.

The activated layer silicates in accordance with the invention can be used, in particular, as fuller's earth materials for the treatment of oils, fats or waxes.

Glyceride oils, waxes and fats and mineral oils pass through one or more adsorptive treatment stages during their refining via inorganic adsorbents. The oil or fat that is to be treated is thereby brought into contact with an inorganic adsorbent at an elevated temperature. In this connection, the adsorbent has the task of freeing the oil from substances that are disadvantageous in subsequent processes or for storage, such as e.g. pigments, phospholipids, materials that produce turbidity, metals, free fatty acids, oxidized compounds, etc. In order to do this, the adsorbent requires adsorptive properties, in order to permit e.g. the removal of phospholipids or chlorophyll materials, and catalytic properties for e.g. degrading dyes or peroxide compounds that are contained in the oil.

Because of their advantageous properties, especially their high specific surface area and their sorption capacity and ion exchange capacity, the activated layer silicates that are prepared in accordance with the invention can also find use in other sectors.

The layer silicates that are listed in Ullmann's Encyklopädie der technischen Chemie ["Encyclopedia of industrial Chemistry"], Volume 21, pages 370-375 (1982) are included among the layer silicates that are usable in the process in accordance with the invention. In particular,

use can be made of natural and synthetic clay minerals that are capable of being activated, such as e.g. the smectites - including montmorillonite, beidellite, nontronite, wolchonskoite, stevensite, hectorite, swinefordite, saponite and sauconite - along with vermiculites, illites, mixed layer minerals, palygorskite (attapulgite) and sepiolite. The latter two materials are also designated hormites. The clay minerals can be present in their H form, their alkali metal form or their alkaline earth form.

In accordance with a preferred form of embodiment in accordance with the invention, the layer silicate is a triple layer silicate, e.g. a naturally occurring smectitic clay, especially a bentonite clay or a palygorskite clay or mixtures thereof.

Palygorskite clays comprise attapulgite clays that are also known as attalpulgus clays, or Georgia fuller's earths. As a rule, these clays consist primarily of the mineral attapulgite, i.e. a crystalline, hydrated magnesium aluminum silicate, but they can also contain considerable quantities of other minerals, such as e.g. bentonite (montmorillonite), calcium carbonate, quartz and feldspar and, in many cases, sepiolite. The preferred clays contain at least 10% by weight, and up to 90% by weight, of attapulgite and, preferably, up to 20 to 60% by weight thereof.

Non-calcined, naturally occurring mixtures of palygorskite clay and calcium bentonite clay are especially preferred. Such natural mixtures can contain pyrites that can serve as a substrate for sulfur-oxidizing bacteria and iron-oxidizing bacteria such as Thiobacillus ferrooxidans.

An attapulgite/bentonite mixture of clays is used in accordance with an especially preferred form of embodiment.

The microorganisms that are used for activation in accordance with the invention are bacteria, archaebacteria or fungi e.g. of the Aspergillus, Acidianus, Acidimicrobium, Acidiphilium, Acidobacterium, Acidocella, Alicyclobacillus, Leptospirillium, Metallosphaera, Picrophylus, Sarcina, Stygiolobus, Sulfobacillus, Sulfolobus, Thermoplasma, Thiobacillus and Thiomonas strains. In addition to the acid-producing bacteria that are preferred here - especially the so-called sulfuric acid bacteria - use can also be made of nitric acid bacteria and acetic acid bacteria

as well as microorganisms that produce oxalic acid, citric acid, gluconic acid or other organic acids.

The use of bacteria that oxidize pyrites is especially advantageous when the layer silicate that is used already contains pyrites, so that this nutrient substance for the bacteria does not have to be added. In addition, it has been found that some naturally occurring bentonite/attapulgite clay mixtures already naturally contain Thiobacillus ferrooxidans and Thiobacillus thiooxidans in small quantities, and these can be induced to activate the layer silicate.

The last two types of bacteria that were designated are strongly chemolithoautotrophic, i.e. their growth cannot be stimulated by providing organic materials, such as nutrient substances or vitamins. Both belong to the group of acidophilic bacteria and they prefer pH values around 2 and temperatures of around 30°C.

Use can be made of both wild type strains of the microorganisms and also strains that have been cultivated in the laboratory (e.g. Thiobacillus thiooxidans DSMZ-11478; Aspergillus niger DSMZ-823; see the DSMZ catalog, 1998). Prior cultivation of the microorganisms offers the advantage that adaptation can be carried out in terms of the conditions that have been selected for the activation of the layer silicate. In addition, the microorganisms can be selected conventionally in terms of advantageous properties (e.g. rapid growth under the conditions that have been selected for activation).

In the case of using aerobic microorganisms (such as, for example, T. thiooxidans, T. ferrooxidans and A. niger), an adequate supply of oxygen has to be ensured during activation of the layer silicate. This can be ensured, for example, by regular mixing in with the layer silicate (every 1 to 7 days) and avoiding excessive compaction. The process of mixing in also encourages uniform distribution, more rapid multiplication and higher metabolic efficiency of the microorganisms; as a result, activation of the layer silicate can be influenced in a positive manner.

It has been found that some naturally occurring bentonite/attapulgite clay mixtures already contain low concentrations of T. thiooxidans and T. ferrooxidans. As a rule, however, it is preferable to add the bacteria to the layer silicate. This can take place, for example, by spraying with a concentrated bacterial culture or by mixing with an inoculant material that has a high concentration of bacteria. The following are suitable, in particular, as the inoculant material: a sample of the layer silicate, which is to be activated or which, as the case may be, has already been bacterially activated, or a bacterial substrate (such as sulfur or pyrites) with a concentration of 10^2 - 10^{10} bacteria/g of inoculant material, or mixtures thereof.

In accordance with one form of embodiment in accordance with the invention, a nutrient substrate in the form of sulfur, pyrites and/or a nutrient salt solution is added to the layer silicate for better growth of the microorganisms. The addition of S is required, in particular, when use is to be made of purely sulfur-oxidizing microorganisms and the layer silicate naturally contains no source of energy (sulfur source or iron source, respectively) that is capable of being utilized by the microorganism in question.

The treatment of the layer silicate with the microorganisms is carried out under conditions that are favorable for the microorganism (or microorganisms) in question. The technical expert will be familiar with these conditions on the basis of the relevant prior art.

Thus, for example, one has to ensure that the microorganisms receive an adequate supply of nutrient substances (e.g. N, K, Ca, Mg, P), vitamins, metabolic substrates, gases (e.g. oxygen, carbon dioxide). If the material that is to be activated naturally contains too little of the substances that are required by the microorganisms that are used in each case, then these can be added to the material.

In the case of using T. ferrooxidans and T. thiooxidans, nutrient salts and/or energy-supplying substrates (e.g. sulfur, pyrites) can be added to the material that is to be activated. Since the designated bacteria are, of necessity, chemolithoautotrophic organisms, these cannot be stimulated via an addition of organic substrates, vitamins or nutrient substances. In some cases, an excessive supply of nutrient salts, in particular, had a negative influence on the activity of the

microorganisms.

In the case of utilizing Aspergillus niger, use can be made, for example, of glucose, sucrose or molasses as the substrate.

An adequate water content of the medium or, as the case may be, the layer silicate, and maintenance of a suitable temperature have to be borne in mind as well. Thus, for example, temperatures of approximately 20 to approximately 35°C and, especially, approximately 30°C, and a water content of more than approximately 15% by weight and, in particular, approximately 60 to 70% by weight based on the layer silicate are preferred when using T. ferrooxidans, T. thiooxidans or A. niger. Aqueous suspensions can also be used.

In order to control the water content when carrying out activation in the open air, it can be necessary to guard against intensive irrigation via rainfall (e.g. by storing under a roof, or by applying air-permeable agricultural foils) or to irrigate artificially in the case of dry weather.

The optimum duration of the activation process in individual cases is dependent on the microorganisms that are used and on the nature of the layer silicate that is used and on the ambient conditions, and it can be ascertained with ease by the technical expert via empirical trials on the basis of the decolorizing activity of the layer silicates that have undergone treatment. In general, microbial activation of the layer silicate is carried out over a period of 1 to 150 days. In some cases, however, it can be advantageous to carry out microbial activation over a longer period of time, e.g. for approximately one year. The duration of the activation process can frequently be shortened by carrying out mechanical size reduction of the pieces of the layer silicate after e.g. one week in order to generate new surfaces for bacterial colonization.

In accordance with one form of embodiment, the process in accordance with the invention comprises the following steps: fresh raw clay is broken up into pieces of the order of approximately 2 cm in size; as a result, a large surface area is generated that is accessible to the microorganisms and the air. The clay is then mixed or kneaded with 5-20% of inoculant clay with a high concentration of bacteria and, as a result, colonization with microorganisms is

accelerated. Piles or stacks are formed that are approximately 10-50 cm high. Excessively high heaping up or compaction would prevent effective aeration. The temperature and water content of the clay are checked and kept as constant as possible during microbial activation. Regular and adequate mixing together and aeration of the clay can take place, for example, via a rotary hoe every 1-8 days. The reduction of the pH value can be measured after drying the clay, or directly via a soil pH meter. Part of the activated clay is used as the inoculant clay after the desired degree of activation has been reached (generally between pH 2 and pH 4). The remainder is dried and ground, whereby the microorganisms that are contained in the clay are also killed off.

The use of microorganisms for the activation of layer silicates is also the subject of the invention.

An especially advantageous feature of the process in accordance with the invention is that one does not have to work with corrosive acids that cause intense burns and endanger natural water systems. Thus it [the process] is superior to the prior art from the standpoints of operational safety and environmental protection. Since only very cheap raw materials, such as pyrites (which is optionally already present in the layer silicate), sulfur and water, are used for microbial activation, the process in accordance with the invention is superior from the economic standpoint as well. Thus the pyrites does not need to be purified completely in the form of a hard accompanying mineral [translator: the meaning of this sentence is not completely clear to me].

It has been found that the pH value or, as the case may be, the quantity of acid that is set free by the microorganisms does not correlate strictly with the activity of the layer silicate that is to be treated. This suggests that microbial activation in accordance with the invention differs from purely acidic activation, and that further metabolic products are involved.

Free iron ions, which are present in the activated layer silicate and which can interfere with the decolorization of oil, are complexed by the Fe-chelating materials that are produced by the microorganisms that are used. In addition, many of the microbial organic acids complex multivalent cations, such as Al^{3+} or Ca^{2+}, and, as a result, these are removed from the equilibrium and activation of the layer silicate is favored.

In addition, free phosphate is incorporated into organic compounds via the microorganisms and these organic compounds and the microorganisms adhere firmly to the layer silicate so that phosphate contamination is reduced in the oil that is to be decolorized.

In addition, interfering cations can be fixed (so-called "bio-accumulation") via absorption into the microorganisms. The accumulation of Cd^{2+}, Co^{2+}, Cu^{2+}, Cr^{3+}, Fe^{3+} and Ni^{2+} has been demonstrated in the case of thio-bacteria, and the accumulation of radionuclides, Co^{2+}, Cu^{2+} and Zn^{2+} has been demonstrated in the case of A. niger.

The surface of the mineral is also rendered [more] hydrophobic by the microorganisms. The increased hydrophobicity of the surface of the layer silicate can lead to better wetting of the particles of fuller's earth by the oils that are to be decolorized.

Additional advantages can be traced back to uniform, in situ activation by the microorganisms, and to the gradual release of acids or, as the case may be, metabolic products. Since the microorganisms that are preferably used, such as T. ferrooxidans and T. thiooxidans, no longer grow at excessively low pH values (e.g. less than 1.5), an excessively high residual acid concentration, which is disadvantageous for the decolorization of oils, can also be avoided in the activated layer silicate. As soon as the pH value has declined too much, the microorganisms terminate their growth and the production of acid. The microorganisms thus act like an internal regulating system for the activation of the layer silicate. Local pH peaks, which arise with ease in the case of an external addition of acid, can also be avoided in this way.

The degradation of pyrites, which is contained in the raw clay and which is utilized by T. ferrooxidans as a nutrient substrate during activation, can be advantageous in some applications of the activated layer silicates since pyrites exhibits an abrasive action during the grinding of the fuller's earth.

The examples of embodiments that follow below will demonstrate the invention and the advantages relative to the prior art. However, the invention is not limited to the examples below.

Examples

Examples 1 - 6

Freshly degraded attapulgite (palygorskite)/bentonite clay with a solids content of 44% was used as the starting material for comparison Examples 1 and 2 and for Examples 3 - 6. According to x-ray phase analysis and chemical composition [tests], this clay comprises 55% palygorskite, 35% Ca montmorillonite, 5% quartz, 3% calcite and 1.5% pyrites. The clay was mechanically reduced to a grain size of approximately 2 cm. This clay, which had been treated in that way, is designated raw clay A in the following sections.

Example 1 (comparison)

A sample of the raw clay A was dried at 80°C to give a water content of 15% and ground to give a sieve residue (64 µm) of 25%; it was then dried at 110°C to give a water content of 8%. After suspending 8 parts of the sample in 100 parts of water, the pH value of the sample was measured by means of a pH measurement electrode.

Decolorization experiments were carried out using rape-seed oil (100 g of oil; 0.75 g of sample; p = 30 mbar; T = 110°C; t = 30 minutes) and soy oil (100 g of oil; 0.50 g of sample; p = 30 mbar; T = 100°C; t = 30 minutes) in order to ascertain the activity of the sample for decolorizing vegetable oil. The decolorizing activity was assessed on the basis of red values, which were ascertained by means of a Lovibond color meter, and on the basis of the spectrophotometrically measured chlorophyll concentrations. In both cases, smaller values signify higher decolorizing activity. The results are indicated in Table I (decolorization of rape-seed oil) and Table II (decolorization of soy oil); in every case, the numerical data are average values from three experiments.

Example 2 (comparison)

340 g of raw clay A were intensively kneaded for 5 minutes with 50 ml of water and 3 g of

concentrated sulfuric acid. The product was then dried and ground as in comparison Example 1. The measurement of the pH value and that of the decolorizing activity took place as in comparison Example 1. The results are indicated in Tables I and II.

Example 3

340 g of raw clay A were mixed with 110 ml of water and homogenized with the help of a sterile wooden spatula. Incubation then took place in a climatic chamber at a constant 30°C and 100% atmospheric humidity. Homogenization of the sample was repeated once per week. Each week, a small aliquot portion was taken out and the pH value was measured as described in comparison Example 1.

When no further change in the pH value could be detected (68 days), the product was dried and ground as in comparison Example 1. The measurement of the pH value and that of the decolorizing activity for rape-seed oil took place as in comparison Example 1. The results are indicated in Table I.

Example 4

340 g of raw clay A were incubated as described in Example 3. After 42 days, 45 g of this material were taken out and mixed with 340 g of fresh raw clay A and 110 ml of water and homogenized with the help of a sterile wooden spatula. Incubation then took place in a climatic chamber at a constant 30°C and 100% atmospheric humidity. Homogenization of the sample was repeated once per week. Each week, a small aliquot portion was taken and the pH value was measured as described in comparison Example 1.

When no further change in the pH value could be detected (21 days), the product was dried and ground as in comparison Example 1. The measurement of the pH value and that of the decolorizing activity for rape-seed oil took place as in comparison Example 1. The results are indicated in Table I.

Example 5

1 ml of a suspension of bacteria (Thiobacillus ferrooxidans; DSMZ strain 11477) and 7.0 g of pyrites (particle size < 64 µm) were added to 100 ml of a nutrient medium comprising 2.00 g/l of $(NH_4)_2SO_4$, 0.50 g/l of K_2HPO_4, 0.50 g/l of $MgSO_4.7H_2O$, 0.10 g/l of KCl and 0.01 g/l of $Ca(NO_3)_2$, whereby this nutrient medium had been adjusted to a pH value of 2 using sulfuric acid. A stream of air was led through this mixture for sufficiently long at 30°C until the pH value of the solution had fallen to 1.75. The pyrites was separated from the solution by means of centrifugation at 1,500 g (5 minutes) and then suspended in 100 ml of water and centrifuged again. The bacterial cells were harvested from the combined centrifuged liquids by centrifugation at 8,000 g (15 minutes) and then they were suspended in 110 ml of water.

340 g of raw clay were treated in an autoclave under standard conditions (T = 120°C; p = 2 bar; t = 30 minutes) in order to kill off the microorganisms that were contained in the raw clay. The raw clay was then mixed with 110 ml of bacterial suspension and homogenized with the help of a sterile wooden spatula; incubation then took place in a climatic chamber at a constant 30°C and 100% atmospheric humidity. Homogenization of the sample was repeated once per week. Each week, a small aliquot portion was taken out and the pH value was measured as described in comparison Example 1. When no further change in the pH value could be detected (56 days), the product was dried and ground as in comparison Example 1. The measurement of the pH value and that of the decolorizing activity for rape-seed oil took place as in comparison Example 1. The results are indicated in Table I.

Example 6

Spores of Aspergillus niger (DSMZ strain 823) were added to 110 ml of a sterile nutrient medium comprising 1.60 g/l of NH_4NO_3, 0.30 g/l of K_2HPO_4, 0.20 g/l of $MgSO_4.7H_2O$, and 6.50 g of glucose, whereby this nutrient medium had been adjusted to a pH value of 4.0 using sulfuric acid. A stream of air was led through this mixture for sufficiently long at 30°C until the pH value of the solution had fallen to 3.0.

340 g of raw clay were treated in an autoclave under standard conditions (T = 120°C; p = 2 bar; t = 30 minutes) in order to kill off the microorganisms that were contained in the raw clay. The raw clay was then mixed with the fungal suspension and homogenized with the help of a sterile wooden spatula; incubation then took place in a climatic chamber at a constant 30°C and 100% atmospheric humidity. Homogenization of the sample was repeated daily. Each week, a small aliquot portion was taken out and the pH value was measured as described in comparison Example 1. When no further change in the pH value could be detected (21 days), the product was dried and ground as in comparison Example 1. The measurement of the pH value and that of the decolorizing activity for soy oil took place as in comparison Example 1. The results are indicated in Table II.

Examples 7 - 11

South American bentonite with a solids content of 60% was used as the starting material for comparison Examples 7 - 9 and for Examples 10 - 11. According to x-ray phase analysis and chemical composition [tests], this clay comprises 90% disordered smectite/illite mixed layer minerals, 2% quartz, 2% calcite and 6% feldspar. The clay was mechanically reduced to a grain size of approximately 2 cm. This clay, which had been treated in that way, is designated raw clay B in the following sections.

Example 7 (comparison)

A sample of the raw clay B was dried at 80°C to give a water content of 15% and ground to give a sieve residue (64 µm) of 25%; it was then dried at 100°C to give a water content of 8%. After suspending 8 parts of the sample in 100 parts of water, the pH value of the sample was measured by means of a pH measurement electrode.

Decolorization experiments were carried out using rape-seed oil (100 g of oil; 0.75 g of sample; p = 30 mbar; T = 110°C; t = 30 minutes) and soy oil (100 g of oil; 0.50 g of sample; p = 30 mbar; T = 100°C; t = 30 minutes) in order to ascertain the activity of the sample for decolorizing vegetable oil. The decolorizing activity was assessed on the basis of red values, which were

ascertained by means of a Lovibond color meter, and on the basis of the spectrophotometrically measured chlorophyll concentrations. In both cases, smaller values signify higher decolorizing activity. The results are indicated in Table I (decolorization of rape-seed oil) and Table II (decolorization of soy oil); in every case, the numerical data are average values from three experiments.

Example 8 (comparison)

250 g of raw clay B were intensively kneaded for 5 minutes with 120 ml of water and 3 g of concentrated sulfuric acid. The product was then dried and ground as in comparison Example 7. The measurement of the pH value and that of the decolorizing activity for soy oil and rape-seed oil took place as in comparison Example 7. The results are indicated in Tables I and II.

Example 9 (comparison)

250 g of raw clay B were mixed with 125 ml of water and homogenized with the help of a sterile wooden spatula. Incubation then took place in a climatic chamber at a constant 30°C and 100% atmospheric humidity. Homogenization of the sample was repeated once per week. Each week, a small aliquot portion was taken out and the pH value was measured as described in comparison Example 7.

After 68 days, the product was dried and ground as in comparison Example 7. The measurement of the pH value and that of the decolorizing activity for soy oil and rape-seed oil took place as in comparison Example 7. The results are indicated in Tables I and II.

Example 10

340 g of raw clay A were incubated as described in Example 3.

After 42 days, 46 g of this material were taken out and mixed with 250 g of raw clay B, 7 g of pyrites and 125 ml of water and homogenized with the help of a sterile wooden spatula.

Incubation then took place in a climatic chamber at a constant 30°C and 100% atmospheric humidity. Homogenization of the sample was repeated once per week. Each week, a small aliquot portion was taken and the pH value was measured as described in comparison Example 7.

When no further change in the pH value could be detected (42 days), the product was dried and ground as in comparison Example 7. The measurement of the pH value and that of the decolorizing activity for soy oil and rape-seed oil took place as in comparison Example 7. The results are indicated in Tables I and II.

Example 11

340 g of raw clay A were incubated as described in Example 3.

After 42 days, 46 g of this material were taken out and mixed with 250 g of raw clay B, 7 g of sulfur and 125 ml of water and homogenized with the help of a sterile wooden spatula. Incubation then took place in a climatic chamber at a constant 30°C and 100% atmospheric humidity. Homogenization of the sample was repeated once per week. Each week, a small aliquot portion was taken and the pH value was measured as described in comparison Example 7.

When no further change in the pH value could be detected (56 days), the product was dried and ground as in comparison Example 7. The measurement of the pH value and that of the decolorizing activity took place as in comparison Example 7. The results are indicated in Tables I and II.

Table I: **Decolorization of rape-seed oil**

	pH	Red value	Chlorophyll A [ppb]	Time [d]	Brief description
Comparison example 1	6.9	5.5	650	0	raw clay A
Comparison example 2	2.8	4.4	300	0	raw clay A + sulfuric acid
Example 3	3.4	4.2	225	68	raw clay A incubated
Example 4	3.4	4.1	220	21	raw clay A + inoculant clay
Example 5	3.4	4.3	240	56	raw clay A inoculated with DSMZ strain
Comparison Example 7	8.4	7.8	800	0	raw clay B
Comparison Example 8	2.3	4.6	310	0	raw clay B + sulfuric acid
Comparison Example 9	8.2	7.8	790	68	raw clay B incubated
Example 10	2.6	4.2	220	42	raw clay B + pyrites + inoculant clay
Example 11	2.8	4.4	290	56	raw clay B + sulfur + inoculant clay

Table II: **Decolorization of soy oil**

	pH	Red value	Chlorophyll A [ppb]	Time [d]	Brief description
Comparison Example 1	6.9	6.4	290	0	raw clay A
Comparison Example 2	2.8	6.7	180	0	raw clay A + sulfuric acid
Example 6	3.4	6.0	170	21	raw clay A inoculated with A. niger
Comparison Example 7	8.4	14.0	680	0	raw clay B
Comparison Example 8	2.3	9.2	170	0	raw clay B + sulfuric acid
Comparison Example 9	8.2	13.8	690	68	raw clay B incubated
Example 10	2.6	6.2	150	42	raw clay B + pyrites + inoculant clay
Example 11	2.8	6.5	170	56	raw clay B + sulfur + inoculant clay

As can be seen from Table I, it was possible to induce the wild strain populations of T. ferrooxidans and T. thiooxidans, which are present in raw clay A, to activate the layer silicate by means of suitable conditions. The activated fuller's earth that was obtained exhibited good results for the decolorization of rape-seed oil and surpassed both raw clay A (comparison Example 1) and a fuller's earth (comparison Example 2), which was prepared in accordance with the prior art by activation with concentrated sulfuric acid, in terms of the red values and the removal of chlorophyll.

As Example 4 shows, the duration of activation using raw clay A can be drastically shortened by mixing it with inoculant clay, which already contains large wild strain populations of T. ferrooxidans and T. thiooxidans, with equally good decolorizing activity for rape-seed oil.

A further addition of a nutrient salt solution to the raw clay samples in Examples 3 and 4 did not lead to increased activity of the bacteria in the first 30 days. This can be traced back to the feature that the wild strain populations of T. ferrooxidans and T. thiooxidans, which are present in raw clay A, had become adapted to very low quantities of nutrient salt over a period of many generations.

Example 5 shows that, in addition to wild strains, cultivated strains of T. ferrooxidans are also suitable for the activation of the layer silicate in raw clay A. The longer duration of the activation process in comparison to Example 4 can be traced back to the feature that the strains, which have become adapted to higher nutrient salt concentrations, first have to become adapted to the lower concentrations in raw clay A.

As Example 6 documents, it was possible to undertake activation of the layer silicates, which were contained in raw clay A, by means of the Aspergillus niger fungus. Glucose as the nutrient source had to be added to the raw clay in this case. It can be seen from Table II that, relative to comparison Example 1, Example 6 shows considerably better removal of chlorophyll and a better red value for the decolorization of soy oil. By contrast, treatment of raw clay A with sulfuric acid in accordance with the prior art (comparison Example 2) results in almost equally good absorption of chlorophyll but a worsening of the red value, whereby this can be traced back to the

low pH value of the adsorption agent and to the high proportion of residual acid that is associated therewith.

Examples 10 and 11 show that, in addition to pyrites-containing attapulgite earths, other layer silicates are likewise capable of being activated via the use of microorganisms.

As far as the removal of red components and, in particular, chlorophyll is considered, comparison Example 7 shows very bad results for the decolorization of both rape-seed oil and soy oil. According to comparison Example 8, a distinct improvement in decolorization activity is possible via a treatment with sulfuric acid that corresponds to the prior art (see Tables I and II).

If raw clay B is merely incubated (comparison Example 9), then no improvement in decolorizing activity occurs. This can be traced back to the deficiency in energy-supplying accompanying substances (such as e.g. pyrites) in raw clay B and the absence, which is related thereto, of microorganisms (e.g. T. ferrooxidans) that utilize these accompanying substances.

Example 10 shows that activation of the layer silicate in raw clay B can be achieved by an addition of pyrites as the supplier of energy together with inoculant populations of T. ferrooxidans and T. thiooxidans from raw clay A and subsequent incubation. In comparison to raw clay B (comparison Example 7) and raw clay B that had been activated in accordance with the prior art (comparison Example 8), a distinct improvement in decolorizing action is found both in rape-seed oil (Table I) and in soy oil (Table II). The duration of the activation process has been prolonged relative to e.g. Example 4. It is probable that the bacteria, which have become adapted to the conditions in raw clay A, first have to become adapted to the ambient conditions that prevail in raw clay B.

It can be seen from Example 11, that activation of the layer silicate in raw clay B is also possible by supplying elemental sulfur, followed by inoculation with incubated raw clay A and subsequent incubation. The duration of activation is further prolonged relative to Example 10 because the wild strain populations of T. thiooxidans from raw clay A have to become adapted not only to the changed conditions in raw clay B, but also to the non-adapted energy source. Relative to

comparison Examples 7 and 8, the layer silicate that was activated in accordance with Example 11 exhibits improved activity levels for decolorizing rape-seed oil and soy oil. In comparison to Example 10, lower decolorizing activity was found in the two oils that were investigated; in contrast to this, Example 11 offers the possibility of activation without an addition of pyrites.

Patent claims

1. Process for the activation of layer silicates, whereby acid-producing microorganisms are used for activation.

2. Process in accordance with Claim 1, characterized by the feature that a smectitic clay mineral is used as the layer silicate.

3. Process in accordance with Claim 1 or 2, characterized by the feature that a montmorillonite-containing clay, especially bentonite, is used as the layer silicate.

4. Process in accordance with one of the above claims, characterized by the feature that a palygorskite clay or mixtures comprising palygorskite and bentonite are used as the layer silicate.

5. Process in accordance with one of the above claims, characterized by the feature that sulfur-oxidizing bacteria and/or iron-oxidizing bacteria, especially Thiobacillus ferrooxidans and/or Thiobacillus thiooxidans, are used as the microorganisms.

6. Process in accordance with one of the above claims, characterized by the feature that microorganisms that produce citric acid, especially Aspergillus niger, are used as the microorganisms.

7. Process in accordance with one of the above claims, characterized by the feature that the microorganisms are wild type strains, which occur in the layer silicate, or cultivated strains.

8. Process in accordance with one of the above claims, characterized by the feature that the clay is first broken up into pieces with a size of approximately 0.5 cm to approximately 5 cm,

especially approximately 2 cm.

9. Process in accordance with one of the above claims, characterized by the feature that the layer silicate is mixed with an inoculant material that has a population of 10^2 to 10^{10} bacteria/g of inoculant material.

10. Process in accordance with one of the above claims, characterized by the feature that sulfur, pyrites, glucose, molasses and/or a nutrient salt solution for the microorganisms is added to the layer silicate.

11. Process in accordance with one of the above claims, characterized by the feature that the treatment with the microorganisms is carried out under growth conditions that are favorable for them, especially at approximately 20 to 35°C and with a water content of more than approximately 15% by weight based on the layer silicate.

12. Process in accordance with one of the above claims, characterized by the feature that the clay is mixed thoroughly and aerated several times during activation.

13. Process in accordance with one of the above claims, characterized by the feature that microbial activation is carried out for 1 to approximately 365 days.

14. Activated layer silicates, obtainable in accordance with one of the above claims.

15. Process for decolorizing oils, fats or waxes that comprises contacting the oil with fuller's earth that is obtainable via a process in accordance one of the above Claims 1 through 14.

16. Use of acid-producing microorganisms for the preparation of fuller's earths for the treatment of oils, fats or waxes.

SUMMARY

A process is specified for the microbial activation of layer silicates.

W/Aw

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COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY
(Includes Reference to PCT International Applications)

ATTORNEY'S DOCKET NUMBER
P-1027

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

MICROBIAL ACTIVATION OF LAYER SILICATES

the specification of which (check only one item below):

☑ is attached hereto.

☐ was filed as United States application

Serial No. _____

on _____,

and was amended

on _____ (if applicable).

☐ was filed as PCT international application

Number _____

on _____,

and was amended under PCT Article 19

on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowlege the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

COUNTRY (if PCT indicate PCT)	APPLICATION NUMBER	DATE OF FILING (day month year)	PRIORITY CLAIMED UNDER 35 USC 119
PCT	PCT/EP 99/05711	06 August 1999	☑ YES ☐ NO
Germany	198 50 129.3	30 October 1998	☑ YES ☐ NO
			☐ YES ☐ NO
			☐ YES ☐ NO
			☐ YES ☐ NO

Combined Declaration For Patent Application and Power of Attorney (Continued)

ATTORNEY'S DOCKET NUMBER: P-1027

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowlege the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C 120

U.S. APPLICATIONS		STATUS (Check one)		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED

PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLICATION NO.	PCT FILING DATE	U.S. SERIAL NUMBERS ASSIGNED (if any)			
PCT/EP 99/05711	06 Aug. 1999				

POWER OF ATTORNEY. As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(List name and registration number)*

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon

SIGNATURE OF INVENTOR 201	SIGNATURE OF INVENTOR 202	SIGNATURE OF INVENTOR 203
		March 13, 2001
DATE: 19.03.01	DATE: 28.2.01	DATE:

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